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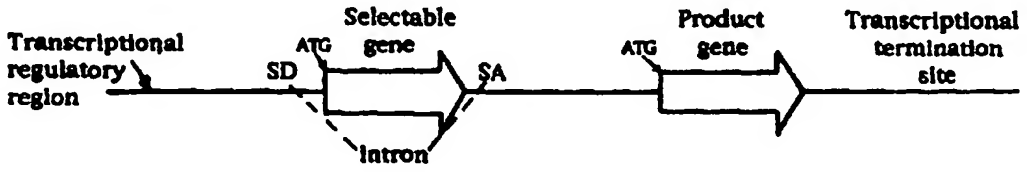

(57) Abstract

A method for selecting recombinant host cells expressing high levels of a desired protein is described. This method utilizes eukaryotic host cells harboring a DNA construct comprising a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene. The selectable gene is positioned within an intron defined by a splice donor site and a splice acceptor site and the selectable gene and product gene are under the transcriptional control of a single transcriptional regulatory region. The splice donor site is generally an efficient splice donor site and thereby regulates expression of the product gene using the transcriptional regulatory region. The transfected cells are cultured so as to express the gene encoding the product in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, whereupon either the desired product is recovered or cells having multiple copies of the product gene are identified.

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METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to a method of selecting for high-expressing host cells, a method of producing a protein of interest in high yields and a method of producing eukaryotic cells having multiple copies of a sequence encoding a protein of interest.

Description of Background and Related Art

The discovery of methods for introducing DNA into living host cells in a functional form has provided the key to understanding many fundamental biological processes, and has made possible the production of important proteins and other molecules in commercially useful quantities.

Despite the general success of such gene transfer methods, several common problems exist that may limit the efficiency with which a gene encoding a desired protein can be introduced into and expressed in a host cell. One problem is knowing when the gene has been successfully transferred into recipient cells. A second problem is distinguishing between those cells that contain the gene and those that have survived the transfer procedures but do not contain the gene. A third problem is identifying and isolating those cells that contain the gene and that are expressing high levels of the protein encoded by the gene.

In general, the known methods for introducing genes into eukaryotic cells tend to be highly inefficient. Of the cells in a given culture, only a small proportion take up and express exogenously added DNA, and an even smaller proportion stably maintain that DNA.

Identification of those cells that have incorporated a product gene encoding a desired protein typically is achieved by introducing into the same cells another gene, commonly referred to as a selectable gene, that encodes a selectable marker. A selectable marker is a protein that is necessary for the growth or survival of a host cell under the particular culture conditions chosen, such as an enzyme that confers resistance to an antibiotic or other drug, or an enzyme that compensates for a metabolic or catabolic defect in the host cell. For example, selectable genes commonly used with eukaryotic cells include the genes for aminoglycoside phosphotransferase (APH), hygromycin phosphotransferase (hyg), dihydrofolate reductase (DHFR), thymidine kinase (tk), neomycin, puromycin, glutamine synthetase, and asparagine synthetase.

The method of identifying a host cell that has incorporated one gene on the basis of expression by the host cell of a second incorporated gene encoding a selectable marker is referred to as cotransfectation (or cotransfection). In that method, a gene encoding a desired polypeptide and a selection gene typically are introduced into the host cell simultaneously, although they may be introduced sequentially. In the case of simultaneous cotransfectation, the gene encoding the desired polypeptide

and the selectable gene may be present on a single DNA molecule or on separate DNA molecules prior to being introduced into the host cells. Wigler *et al.*, Cell, 16:777 (1979). Cells that have incorporated the gene encoding the desired polypeptide then are identified or isolated by culturing the cells under conditions that preferentially allow for the growth or survival of those cells that synthesize the selectable marker encoded by the selectable gene.

The level of expression of a gene introduced into a eukaryotic host cell depends on multiple factors, including gene copy number, efficiency of transcription, messenger RNA (mRNA) processing, stability, and translation efficiency. Accordingly, high level expression of a desired polypeptide typically will involve optimizing one or more of those factors.

For example, the level of protein production may be increased by covalently joining the coding sequence of the gene to a "strong" promoter or enhancer that will give high levels of transcription. Promoters and enhancers are nucleotide sequences that interact specifically with proteins in a host cell that are involved in transcription. Kriegler, Meth. Enzymol., 185:512 (1990); Maniatis *et al.*, Science, 236:1237 (1987). Promoters are located upstream of the coding sequence of a gene and facilitate transcription of the gene by RNA polymerase. Among the eukaryotic promoters that have been identified as strong promoters for high-level expression are the SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Rous sarcoma virus long terminal repeat, and human cytomegalovirus immediate early promoter (CMV).

Enhancers stimulate transcription from a linked promoter. Unlike promoters, enhancers are active when placed downstream from the transcription initiation site or at considerable distances from the promoter, although in practice enhancers may overlap physically and functionally with promoters. For example, all of the strong promoters listed above also contain strong enhancers. Bendig, Genetic Engineering, 7:91 (Academic Press, 1988).

The level of protein production also may be increased by increasing the gene copy number in the host cell. One method for obtaining high gene copy number is to directly introduce into the host cell multiple copies of the gene, for example, by using a large molar excess of the product gene relative to the selectable gene during cotransfectation. Kaufman, Meth. Enzymol., 185:537 (1990). With this method, however, only a small proportion of the cotransfected cells will contain the product gene at high copy number. Furthermore, because no generally applicable, convenient method exists for distinguishing such cells from the majority of cells that contain fewer copies of the product gene, laborious and time-consuming screening methods typically are required to identify the desired high-copy number transfectants.

Another method for obtaining high gene copy number involves cloning the gene in a vector that is capable of replicating autonomously in the host cell. Examples of such vectors include mammalian expression vectors

derived from Epstein-Barr virus or bovine papilloma virus, and yeast 2-micron plasmid vectors. Stephens & Hentschel, Biochem. J., 248:1 (1987); Yates *et al.*, Nature, 313:812 (1985); Beggs, Genetic Engineering, 2:175 (Academic Press, 1981).

Yet another method for obtaining high gene copy number involves gene amplification in the host cell. Gene amplification occurs naturally in eukaryotic cells at a relatively low frequency. Schimke, J. Biol. Chem., 263:5989 (1988). However, gene amplification also may be induced, or at least selected for, by exposing host cells to appropriate selective pressure. For example, in many cases it is possible to introduce a product gene together with an amplifiable gene into a host cell and subsequently select for amplification of the marker gene by exposing the cotransfected cells to sequentially increasing concentrations of a selective agent. Typically the product gene will be coamplified with the marker gene under such conditions.

The most widely used amplifiable gene for that purpose is a DHFR gene, which encodes a dihydrofolate reductase enzyme. The selection agent used in conjunction with a DHFR gene is methotrexate (Mtx). A host cell is cotransfected with a product gene encoding a desired protein and a DHFR gene, and transfectants are identified by first culturing the cells in culture medium that contains Mtx. A suitable host cell when a wild-type DHFR gene is used is the Chinese Hamster Ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub & Chasin, Proc. Nat. Acad. Sci. USA, 77:4216 (1980). The transfected cells then are exposed to successively higher amounts of Mtx. This leads to the synthesis of multiple copies of the DHFR gene, and concomitantly, multiple copies of the product gene. Schimke, J. Biol. Chem., 263:5989 (1988); Axel *et al.*, U.S. Patent No. 4,399,216; Axel *et al.*, U.S. Patent No. 4,634,665. Other references directed to co-transfection of a gene together with a genetic marker that allows for selection and subsequent amplification include Kaufman in Genetic Engineering, ed. J. Setlow (Plenum Press, New York), Vol. 9 (1987); Kaufman and Sharp, J. Mol. Biol., 159:601 (1982); Ringold *et al.*, J. Mol. Appl. Genet., 1:165-175 (1981); Kaufman *et al.*, Mol. Cell Biol., 5:1750-1759 (1985); Kaetzel and Nilson, J. Biol. Chem., 263:6244-6251 (1988); Hung *et al.*, Proc. Natl. Acad. Sci. USA, 83:261-264 (1986); Kaufman *et al.*, EMBO J., 6:87-93 (1987); Johnston and Kucey, Science, 242:1551-1554 (1988); Urlaub *et al.*, Cell, 33:405-412 (1983).

To extend the DHFR amplification method to other cell types, a mutant DHFR gene that encodes a protein with reduced sensitivity to methotrexate may be used in conjunction with host cells that contain normal numbers of an endogenous wild-type DHFR gene. Simonsen and Levinson, Proc. Natl. Acad. Sci. USA, 80:2495 (1983); Wigler *et al.*, Proc. Natl. Acad. Sci. USA, 77:3567-3570 (1980); Haber and Schimke, Somatic Cell Genetics, 8:499-508 (1982).

Alternatively, host cells may be co-transfected with the product gene, a DHFR gene, and a dominant selectable gene, such as a neo^r gene. Kim

and Wold, Cell, 42:129 (1985); Capon *et al.*, U.S. Pat. No. 4,965,199. Transfectants are identified by first culturing the cells in culture medium containing neomycin (or the related drug G418), and the transfectants so identified then are selected for amplification of the DHFR gene and the product gene by exposure to successively increasing amounts of Mtx.

As will be appreciated from this discussion, the selection of recombinant host cells that express high levels of a desired protein generally is a multi-step process. In the first step, initial transfectants are selected that have incorporated the product gene and the selectable gene. In subsequent steps, the initial transfectants are subject to further selection for high-level expression of the selectable gene and then random screening for high-level expression of the product gene. To identify cells expressing high levels of the desired protein, typically one must screen large numbers of transfectants. The majority of transfectants produce less than maximal levels of the desired protein. Further, Mtx resistance in DHFR transformants is at least partially conferred by varying degrees of gene amplification. Schimke, Cell, 37:705-713 (1984). The inadequacies of co-expression of the non-selected gene have been reported by Wold *et al.*, Proc. Natl. Acad. Sci. USA, 76:5684-5688 (1979). Instability of the amplified DNA is reported by Kaufman and Schimke, Mol. Cell Biol., 1:1069-1076 (1981); Haber and Schimke, Cell, 26:355-362 (1981); and Fedespiel *et al.*, J. Biol. Chem., 259:9127-9140 (1984).

Several methods have been described for directly selecting such recombinant host cells in a single step. One strategy involves co-transfecting host cells with a product gene and a DHFR gene, and selecting those cells that express high levels of DHFR by directly culturing in medium containing a high concentration of Mtx. Many of the cells selected in that manner also express the co-transfected product gene at high levels. Page and Sydenham, Bio/Technology, 9:64 (1991). This method for single-step selection suffers from certain drawbacks that limit its usefulness. High-expressing cells obtained by direct culturing in medium containing a high level of a selection agent may have poor growth and stability characteristics, thus limiting their usefulness for long-term production processes. Page and Snyderman, Bio/Technology, 9:64 (1991). Single-step selection for high-level resistance to Mtx may produce cells with an altered, Mtx-resistant DHFR enzyme, or cells that have altered Mtx transport properties, rather than cells containing amplified genes. Haber *et al.*, J. Biol. Chem., 256:9501 (1981); Assaraf and Schimke, Proc. Natl. Acad. Sci. USA, 84:7154 (1987).

Another method involves the use of polycistronic mRNA expression vectors containing a product gene at the 5' end of the transcribed region and a selectable gene at the 3' end. Because translation of the selectable gene at the 3' end of the polycistronic mRNA is inefficient, such vectors exhibit preferential translation of the product gene and require high levels of polycistronic mRNA to survive selection. Kaufman, Meth.

Enzymol., 185:487 (1990); Kaufman, Meth. Enzymol., 185:537 (1990); Kaufman *et al.*, EMBO J., 6:187 (1987). Accordingly, cells expressing high levels of the desired protein product may be obtained in a single step by culturing the initial transfectants in medium containing a selection agent appropriate for use with the particular selectable gene. However, the utility of these vectors is variable because of the unpredictable influence of the upstream product reading frame on selectable marker translation and because the upstream reading frame sometimes becomes deleted during methotrexate amplification (Kaufman *et al.*, J. Mol. Biol., 159:601-621 [1982]; Levinson, Methods in Enzymology, San Diego: Academic Press, Inc. [1990]). Later vectors incorporated an internal translation initiation site derived from members of the picornavirus family which is positioned between the product gene and the selectable gene (Pelletier *et al.*, Nature, 334:320 [1988]; Jang *et al.*, J. Virol., 63:1651 [1989]).

A third method for single-step selection involves use of a DNA construct with a selectable gene containing an intron within which is located a gene encoding the protein of interest. See U.S. Patent No. 5,043,270 and Abrams *et al.*, J. Biol. Chem., 264(24): 14016-14021 (1989). In yet another single-step selection method, host cells are co-transfected with an intron-modified selectable gene and a gene encoding the protein of interest. See WO 92/17566, published October 15, 1992. The intron-modified gene is prepared by inserting into the transcribed region of a selectable gene an intron of such length that the intron is correctly spliced from the corresponding mRNA precursor at low efficiency, so that the amount of selectable marker produced from the intron-modified selectable gene is substantially less than that produced from the starting selectable gene. These vectors help to insure the integrity of the integrated DNA construct, but transcriptional linkage is not achieved as selectable gene and the protein gene are driven by separate promoters.

Other mammalian expression vectors that have single transcription units have been described. Retroviral vectors have been constructed (Cepko *et al.*, Cell, 37:1053-1062 [1984]) in which a cDNA is inserted between the endogenous Moloney murine leukemia virus (M-MuLV) splice donor and splice acceptor sites which are followed by a neomycin resistance gene. This vector has been used to express a variety of gene products following retroviral infection of several cell types.

With the above drawbacks in mind, it is one object of the present invention to increase the level of homogeneity with regard to expression levels of stable clones transfected with a product gene of interest, by expressing a selectable marker (DHFR) and the protein of interest from a single promoter.

It is another object to provide a method for selecting stable, recombinant host cells that express high levels of a desired protein product, which method is rapid and convenient to perform, and reduces the numbers of transfected cells which need to be screened. Furthermore, it is

an object to allow high levels of single and two unit polypeptides to be rapidly generated from clones or pools of stable host cell transfectants.

It is an additional object to provide expression vectors which bias for active integration events (*i.e.* have an increased tendency to generate transformants wherein the DNA construct is inserted into a region of the genome of the host cell which results in high level expression of the product gene) and can accommodate a variety of product genes without the need for modification.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to a DNA construct (DNA molecule) alternative terminology comprising a 5' transcriptional initiation site and a 3' transcriptional termination site, a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene positioned within an intron defined by a splice donor site and a splice acceptor site. The splice donor site preferably comprises an effective splice donor sequence as herein defined and thereby regulates expression of the product gene using the transcriptional regulatory region.

In another embodiment, the invention provides a method for producing a product of interest comprising culturing a eukaryotic cell which has been transfected with the DNA construct described above, so as to express the product gene and recovering the product.

In a further embodiment, the invention provides a method for producing eukaryotic cells having multiple copies of the product gene comprising transfecting eukaryotic cells with the DNA construct described above (where the selectable gene is an amplifiable gene), growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene. Preferably transfection of the cells is achieved using electroporation.

After transfection of the host cells, most of the transfectants fail to exhibit the selectable phenotype characteristic of the protein encoded by the selectable gene, but surprisingly a small proportion of the transfectants do exhibit the selectable phenotype, and among those transfectants, the majority are found to express high levels of the desired product encoded by the product gene. Thus, the invention provides an improved method for the selection of recombinant host cells expressing high levels of a desired product, which method is useful with a wide variety of eukaryotic host cells and avoids the problems inherent in existing cell selection technology.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D illustrate schematically various DNA constructs encompassed by the instant invention. The large arrows represent the selectable gene and the product gene, the V formed by the dashed lines shows the region of the precursor RNA internal to the 5' splice donor site (SD) and 3' splice acceptor site (SA) that is excised from vectors that contain a functional SD. The transcriptional regulatory region, selectable gene, product gene and transcriptional termination site are depicted in Figure 1A. Figure 1B depicts the DNA constructs of Example 1. The various splice donor sequences are depicted, *i.e.*, wild type ras splice donor sequence (WT ras), mutant ras splice donor sequence (MUTANT ras) and non-functional splice donor sequence (▲GT). The probes used for Northern blot analysis in Example 1 are shown in Figure 1B. Figure 1C depicts the DNA constructs of Example 2 and Figure 1D depicts the DNA construct of Example 3 used for expression of anti-IgE V_H.

Figure 2 depicts schematically the control DNA construct used in Example 1.

Figures 3A-Q depict the nucleotide sequence (SEQ ID NO: 1) of the DHFR/intron-(WT ras SD)-tPA expression vector of Example 1.

Figure 4 is a bar graph which shows the number of colonies that form in selective medium after electroporation of linearized duplicate miniprep DNA's prepared in parallel from the three vectors shown in Figure 1B (*i.e.* with wild type ras splice donor sequence [WT ras], mutant ras splice donor sequence [MUTANT ras] and non-functional splice donor sequence [▲GT]) and from the control vector that has DHFR under control of SV40 promoter and tPA under control of CMV promoter (see Figure 2). Cells were selected in nucleoside free medium and counted with an automated colony counter.

Figures 5A-C are bar graphs depicting expression of tPA from stable pools and clones generated from the vectors shown in Figure 1B. In Figure 5A greater than 100 clones from each vector transfection were mixed, plated in 24 well plates, and assayed by tPA ELISA at "saturation". In Figure 5B, twenty clones chosen at random derived from each of the vectors were assayed by tPA ELISA at "saturation". In Figure 5C, the pools mentioned in Figure 5A (except the ▲GT pool) were exposed to 200nM Mtx to select for DHFR amplification and then pooled and assayed for tPA expression.

Figures 6A-P depict the nucleotide sequence (SEQ ID NO: 2) of the DHFR/intron-(WT ras SD)-TNFr-IgG expression vector of Example 2.

Figures 7A-B are bar graphs depicting expression of TNFr-IgG using dicistronic or control vectors (see Example 2). Vectors containing TNFr-IgG (but otherwise identical to those described for tPA expression in Example 1) were constructed (see Figure 1C), introduced into dp12.CHO cells by electroporation, pooled, and assayed for product expression before (Figure 7A) and after (Figure 7B) being subjected to amplification in 200nM Mtx.

Figure 8 depicts schematically the DNA construct used for expression of the V_L of anti-IgE in Example 3.

Figures 9A-O depict the nucleotide sequence (SEQ ID NO: 3) of the anti-IgE V_H expression vector of Example 3.

Figures 10A-Q depict the nucleotide sequence (SEQ ID NO: 4) of the anti-IgE V_L expression vector of Example 3.

Figure 11 is a bar graph depicting anti-IgE expression in Example 3. Heavy (V_H) and light (V_L) chain expression vectors were constructed, co-electroporated into CHO cells, clones were selected and assayed for antibody expression. Additionally, pools were established and assessed with regard to expression before and after Mtx selection at 200nM and 1μM.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions:

The "DNA construct" disclosed herein comprises a non-naturally occurring DNA molecule which can either be provided as an isolate or integrated in another DNA molecule *e.g.* in an expression vector or the chromosome of an eukaryotic host cell.

The term "selectable gene" as used herein refers to a DNA that encodes a selectable marker necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. Accordingly, a host cell that is transformed with a selectable gene will be capable of growth or survival under certain cell culture conditions wherein a non-transfected host cell is not capable of growth or survival. Typically, a selectable gene will confer resistance to a drug or compensate for a metabolic or catabolic defect in the host cell. Examples of selectable genes are provided in the following table. See also Kaufman, Methods in Enzymology, 185: 537-566 (1990), for a review of these.

TABLE 1

Selectable Genes and their Selection Agents

Selection Agent	Selectable Gene
Methotrexate	Dihydrofolate reductase
Cadmium	Metallothionein
PALA	CAD
Xyl-A-or adenosine and 2'-deoxycoformycin	Adenosine deaminase
Adenine, azaserine, and coformycin	Adenylate deaminase
6-Azauridine, pyrazofuran	UMP Synthetase
Mycophenolic acid	IMP 5'-dehydrogenase

Mycophenolic acid with limiting xanthine	Xanthine-guanine phosphoribosyltransferase
Hypoxanthine, aminopterin, and thymidine (HAT)	Mutant HGPRTase or mutant thymidine kinase
5-Fluorodeoxyuridine	Thymidylate synthetase
Multiple drugs *e.g.* adriamycin, vincristine or colchicine	P-glycoprotein 170
Aphidicolin	Ribonucleotide reductase
Methionine sulfoximine	Glutamine synthetase
β-Aspartyl hydroxamate or Albizziin	Asparagine synthetase
Canavanine	Arginosuccinate synthetase
α-Difluoromethylornithine	Ornithine decarboxylase
Compactin	HMG-CoA reductase
Tunicamycin	*N*-Acetylglucosaminyl transferase
Borrelidin	Threonyl-tRNA synthetase
Ouabain	Na^+K^+-ATPase

The preferred selectable gene is an amplifiable gene. As used herein, the term "amplifiable gene" refers to a gene which is amplified (*i.e.* additional copies of the gene are generated which survive in intrachromosomal or extrachromosomal form) under certain conditions. The amplifiable gene usually encodes an enzyme (*i.e.* an amplifiable marker) which is required for growth of eukaryotic cells under those conditions. For example, the gene may encode DHFR which is amplified when a host cell transformed therewith is grown in Mtx. According to Kaufman, the selectable genes in Table 1 above can also be considered amplifiable genes. An example of a selectable gene which is generally not considered to be an amplifiable gene is the neomycin resistance gene (Cepko *et al.*, *supra*).

As used herein, "selective medium" refers to nutrient solution used for growing eukaryotic cells which have the selectable gene and therefore includes a "selection agent". Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are exemplary nutrient solutions. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58:44 (1979), Barnes and Sato, Anal. Biochem., 102:255

(1980), U.S. Patent Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Patent Re. 30,985; or U.S. Patent No. 5,122,469, the disclosures of all of which are incorporated herein by reference, may be used as culture media. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The preferred nutrient solution comprises fetal bovine serum.

The term "selection agent" refers to a substance that interferes with the growth or survival of a host cell that is deficient in a particular selectable gene. Examples of selection agents are presented in Table 1 above. The selection agent preferably comprises an "amplifying agent" which is defined for purposes herein as an agent for amplifying copies of the amplifiable gene, such as Mtx if the amplifiable gene is DHFR. See Table 1 for examples of amplifying agents.

As used herein, the term "transcriptional initiation site" refers to the nucleic acid in the DNA construct corresponding to the first nucleic acid incorporated into the primary transcript, *i.e.*, the mRNA precursor, which site is generally provided at, or adjacent to, the 5' end of the DNA construct.

The term "transcriptional termination site" refers to a sequence of DNA, normally represented at the 3' end of the DNA construct, that causes RNA polymerase to terminate transcription.

As used herein, "transcriptional regulatory region" refers to a region of the DNA construct that regulates transcription of the selectable gene and the product gene. The transcriptional regulatory region normally refers to a promoter sequence (*i.e.* a region of DNA involved in binding of RNA polymerase to initiate transcription) which can be constitutive or inducible and, optionally, an enhancer (*i.e.* a *cis*-acting DNA element, usually from about 10-300 bp, that acts on a promoter to increase its transcription).

As used herein, "product gene" refers to DNA that encodes a desired protein or polypeptide product. Any product gene that is capable of expression in a host cell may be used, although the methods of the invention are particularly suited for obtaining high-level expression of a product gene that is not also a selectable or amplifiable gene. Accordingly, the protein or polypeptide encoded by a product gene typically will be one that is not necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. For example, product genes suitably encode a peptide, or may encode a polypeptide sequence of

amino acids for which the chain length is sufficient to produce higher levels of tertiary and/or quaternary structure.

Examples of bacterial polypeptides or proteins include, *e.g.*, alkaline phosphatase and β-lactamase. Examples of mammalian polypeptides or proteins include molecules such as renin; a growth hormone, including human growth hormone, and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; antibodies; chimeric proteins such as immunoadhesins and fragments of any of the above-listed polypeptides.

The product gene preferably does not consist of an anti-sense sequence for inhibiting the expression of a gene present in the host. Preferred proteins herein are therapeutic proteins such as TGF-β, TGF-α, PDGF, EGF, FGF, IGF-I, DNase, plasminogen activators such as t-PA, clotting factors such as tissue factor and factor VIII, hormones such as relaxin and insulin, cytokines such as IFN-γ, chimeric proteins such as TNF receptor IgG immunoadhesin (TNFr-IgG) or antibodies such as anti-IgE.

The term "intron" as used herein refers to a nucleotide sequence present within the transcribed region of a gene or within a messenger RNA precursor, which nucleotide sequence is capable of being excised, or spliced, from the messenger RNA precursor by a host cell prior to translation. Introns suitable for use in the present invention are suitably prepared by any of several methods that are well known in the art, such as purification from a naturally occurring nucleic acid or *de novo* synthesis. The introns present in many naturally occurring eukaryotic genes have been identified and characterized. Mount, Nuc. Acids Res., 10:459 (1982). Artificial introns comprising functional splice sites also have been described. Winey *et al.*, Mol. Cell Biol., 9:329 (1989); Gatermann *et al.*, Mol. Cell Biol., 9:1526 (1989). Introns may be obtained from naturally occurring nucleic acids, for example, by digestion of a naturally occurring nucleic acid with a suitable restriction endonuclease, or by PCR cloning using primers complementary to sequences at the 5' and 3' ends of the intron. Alternatively, introns of defined sequence and length may be prepared synthetically using various methods in organic chemistry. Narang *et al.*, Meth. Enzymol., 68:90 (1979); Caruthers *et al.*, Meth. Enzymol., 154:287 (1985); Froehler *et al.*, Nuc. Acids Res., 14:5399 (1986).

As used herein "splice donor site" or "SD" refers to the DNA sequence immediately surrounding the exon-intron boundary at the 5' end of the intron, where the "exon" comprises the nucleic acid 5' to the intron. Many splice donor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. An "efficient splice donor sequence" refers to a nucleic acid sequence encoding a splice donor site wherein the efficiency of splicing of messenger RNA precursors having the splice donor sequence is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. Examples of efficient splice donor sequences include the wild type (WT) ras splice donor sequence and the GAC:GTAAGT sequence of Example 3. Other efficient splice donor sequences can be readily selected using the techniques for measuring the efficiency of splicing disclosed herein.

The terms "PCR" and "polymerase chain reaction" as used herein refer to the *in vitro* amplification method described in US Patent No. 4,683,195 (issued July 28, 1987). In general, the PCR method involves repeated cycles of primer extension synthesis, using two DNA primers capable of hybridizing preferentially to a template nucleic acid comprising the nucleotide sequence to be amplified. The PCR method can be used to clone specific DNA sequences from total genomic DNA, cDNA transcribed from cellular RNA, viral or plasmid DNAs. Wang & Mark, in PCR Protocols, pp.70-75 (Academic Press, 1990); Scharf, in PCR Protocols, pp. 84-98; Kawasaki & Wang, in PCR Technology, pp. 89-97 (Stockton Press, 1989). Reverse transcription-polymerasechain reaction (RT-PCR) can be used to analyze RNA samples containing mixtures of spliced and unspliced mRNA transcripts. Fluorescently tagged primers designed to span the intron are used to

amplify both spliced and unspliced targets. The resultant amplification products are then separated by gel electrophoresis and quantitated by measuring the fluorescent emission of the appropriate band(s). A comparison is made to determine the amount of spliced and unspliced transcripts present in the RNA sample.

One preferred splice donor sequence is a "consensus splice donor sequence". The nucleotide sequences surrounding intron splice sites, which sequences are evolutionarily highly conserved, are referred to as "consensus splice donor sequences". In the mRNAs of higher eukaryotes, the 5' splice site occurs within the consensus sequence AG:GUAAGU (wherein the colon denotes the site of cleavage and ligation). In the mRNAs of yeast, the 5' splice site is bounded by the consensus sequence :GUAUGU. Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986).

The expression "splice acceptor site" or "SA" refers to the sequence immediately surrounding the intron-exon boundary at the 3' end of the intron, where the "exon" comprises the nucleic acid 3' to the intron. Many splice acceptor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. The preferred splice acceptor site is an efficient splice acceptor site which refers to a nucleic acid sequence encoding a splice acceptor site wherein the efficiency of splicing of messenger RNA precursors having the splice acceptor site is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. The splice acceptor site may comprise a consensus sequence. In the mRNAs of higher eukaryotes, the 3' splice acceptor site occurs within the consensus sequence $(U/C)_{11}$NCAG:G. In the mRNAs of yeast, the 3' acceptor splice site is bounded by the consensus sequence (C/U)AG:. Padgett, *et al.*, *supra*.

As used herein "culturing for sufficient time to allow amplification to occur" refers to the act of physically culturing the eukaryotic host cells which have been transformed with the DNA construct in cell culture media containing the amplifying agent, until the copy number of the amplifiable gene (and preferably also the copy number of the product gene) in the host cells has increased relative to the transformed cells prior to this culturing.

The term "expression" as used herein refers to transcription or translation occurring within a host cell. The level of expression of a product gene in a host cell may be determined on the basis of either the amount of corresponding mRNA that is present in the cell or the amount of the protein encoded by the product gene that is produced by the cell. For example, mRNA transcribed from a product gene is desirably quantitated by northern hybridization. Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, pp. 7.3-7.57 (Cold Spring Harbor Laboratory Press, 1989). Protein encoded by a product gene can be quantitated either by assaying for the biological activity of the protein or by employing assays that are independent of such activity, such as western blotting or radioimmunoassay using antibodies that are capable of reacting with the protein. Sambrook,

et al., Molecular Cloning: A Laboratory Manual, pp. 18.1-18.88 (Cold Spring Harbor Laboratory Press, 1989).

Modes for Carrying Out the Invention

Methods and compositions are provided for enhancing the stability and/or copy number of a transcribed sequence in order to allow for elevated levels of a RNA sequence of interest. In general, the methods of the present invention involve transfecting a eukaryotic host cell with an expression vector comprising both a product gene encoding a desired polypeptide and a selectable gene (preferably an amplifiable gene).

Selectable genes and product genes may be obtained from genomic DNA, cDNA transcribed from cellular RNA, or by *in vitro* synthesis. For example, libraries are screened with probes (such as antibodies or oligonucleotides of about 20-80 bases) designed to identify the selectable gene or the product gene (or the protein(s) encoded thereby). Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the selectable gene or product gene is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues known to contain the selectable gene or product gene. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide generally is labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ^{32}P- labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Sometimes, the DNA encoding the selectable gene and product gene is preceded by DNA encoding a signal sequence having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the expression vector, or it may be a part of the selectable gene or product gene that is inserted into the expression vector. If a heterologous signal sequence is used, it preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native signal sequence

of the protein of interest is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal. The DNA for such precursor region is ligated in reading frame to the selectable gene or product gene.

As shown in Figure 1A, the selectable gene is generally provided at the 5' end of the DNA construct and this selectable gene is followed by the product gene. Therefore, the full length (non-spiced) message will contain DHFR as the first open reading frame and will therefore generate DHFR protein to allow selection of stable transfectants. The full length message is not expected to generate appreciable amounts of the protein of interest as the second AUG in a dicistronic message is an inefficient initiator of translation in mammalian cells (Kozak, J. Cell Biol., 115: 887-903 [1991]).

The selectable gene is positioned within an intron. Introns are noncoding nucleotide sequences, normally present within many eukaryotic genes, which are removed from newly transcribed mRNA precursors in a multiple-step process collectively referred to as splicing.

A single mechanism is thought to be responsible for the splicing of mRNA precursors in mammalian, plant, and yeast cells. In general, the process of splicing requires that the 5' and 3' ends of the intron be correctly cleaved and the resulting ends of the mRNA be accurately joined, such that a mature mRNA having the proper reading frame for protein synthesis is produced. Analysis of a variety of naturally occurring and synthetically constructed mutant genes has shown that nucleotide changes at many of the positions within the consensus sequences at the 5' and 3' splice sites have the effect of reducing or abolishing the synthesis of mature mRNA. Sharp, Science, 235:766 (1987); Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986); Green, Ann. Rev. Genet., 20:671 (1986). Mutational studies also have shown that RNA secondary structures involving splicing sites can affect the efficiency of splicing. Solnick, Cell, 43:667 (1985); Konarska, *et al.*, Cell, 42:165 (1985).

The length of the intron may also affect the efficiency of splicing. By making deletion mutations of different sizes within the large intron of the rabbit beta-globin gene, Wieringa, *et al.* determined that the minimum intron length necessary for correct splicing is about 69 nucleotides. Cell, 37:915 (1984). Similar studies of the intron of the adenovirus E1A region have shown that an intron length of about 78 nucleotides allows correct splicing to occur, but at reduced efficiency. Increasing the length of the intron to 91 nucleotides restores normal splicing efficiency, whereas truncating the intron to 63 nucleotides abolishes correct splicing. Ulfendahl, *et al.*, Nuc. Acids Res., 13:6299 (1985).

To be useful in the invention, the intron must have a length such that splicing of the intron from the mRNA is efficient. The preparation of introns of differing lengths is a routine matter, involving methods well known in the art, such as *de novo* synthesis or *in vitro* deletion

mutagenesis of an existing intron. Typically, the intron will have a length of at least about 150 nucleotides, since introns which are shorter than this tend to be spliced less efficiently. The upper limit for the length of the intron can be up to 30 kB or more. However, as a general proposition, the intron is generally less than about 10 kB in length.

The intron is modified to contain the selectable gene not normally present within the intron using any of the various known methods for modifying a nucleic acid *in vitro*. Typically, a selectable gene will be introduced into an intron by first cleaving the intron with a restriction endonuclease, and then covalently joining the resulting restriction fragments to the selectable gene in the correct orientation for host cell expression, for example by ligation with a DNA ligase enzyme.

The DNA construct is dicistronic, *i.e.* the selectable gene and product gene are both under the transcriptional control of a single transcriptional regulatory region. As mentioned above, the transcriptional regulatory region comprises a promoter. Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255:2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7:149 [1968]; and Holland, Biochemistry, 17:4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Expression control sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide.

Product gene transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the product gene, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209:1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78:7398-7402 (1981). The immediate early promoter of the human cytomegalovirus (CMV) is conveniently obtained as a *Hin*dIII E restriction fragment. Greenaway *et al.*, Gene, 18:355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79:5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

Preferably the transcriptional regulatory region in higher eukaryotes comprises an enhancer sequence. Enhancers are relatively orientation and position independent having been found 5' (Lainins *et al.*, Proc. Natl. Acad. Sci. USA, 78:993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., 3:1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33:729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4:1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer (CMV), the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the product gene, but is preferably located at a site 5' from the promoter.

The DNA construct has a transcriptional initiation site following the transcriptional regulatory region and a transcriptional termination region following the product gene (see Figure 1A). These sequences are provided in the DNA construct using techniques which are well known in the art.

The DNA construct normally forms part of an expression vector which may have other components such as an origin of replication (*i.e.*, a nucleic acid sequence that enables the vector to replicate in one or more selected host cells) and, if desired, one or more additional selectable gene(s). Construction of suitable vectors containing the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA

fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

Generally, in cloning vectors the origin of replication is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known. The 2μ plasmid origin of replication is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

For analysis to confirm correct sequences in plasmids constructed, plasmids from the transformants are prepared, analyzed by restriction, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., 9:309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, 65:499 (1980).

The expression vector having the DNA construct prepared as discussed above is transformed into a eukaryotic host cell. Suitable host cells for cloning or expressing the vectors herein are yeast or higher eukaryote cells.

Eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing the product gene. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *S. pombe* [Beach and Nurse, Nature, 290:140 (1981)], *Kluyveromyces lactis* [Louvencourt *et al.*, J. Bacteriol., 737 (1983)], *yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070], *Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, 76:5259-5263 (1979)], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., 112:284-289 (1983); Tilburn *et al.*, Gene, 26:205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81:1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., 4:475-479 (1985)].

Suitable host cells for the expression of the product gene are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda*

(caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosphila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, 6:47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, 315:592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the product gene. During incubation of the plant cell culture with *A. tumefaciens*, the product gene is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the product gene. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 [1980]); dp12.CHO cells (EP 307,247 published 15 March 1989); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) may be used. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130:946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

In the preferred embodiment the DNA is introduced into the host cells using electroporation. See Andreason, J. Tiss. Cult. Meth., 15:56-62 (1993), for a review of electroporation techniques useful for practicing the instantly claimed invention. It was discovered that electroporation techniques for introducing the DNA construct into the host cells were preferable over calcium phosphate precipitation techniques insofar as the latter could cause the DNA to break up and forming concantemers.

The mammalian host cells used to express the product gene herein may be cultured in a variety of media as discussed in the definitions section above. The media contains the selection agent used for selecting transformed host cells which have taken up the DNA construct (either as an intra- or extra-chromosomal element). To achieve selection of the transformed eukaryotic cells, the host cells may be grown in cell culture plates and individual colonies expressing the selectable gene (and thus the product gene) can be isolated and grown in growth medium until the nutrients are depleted. The host cells are then analyzed for transcription and/or transformation as discussed below. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescens, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the

formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75:734-738 (1980).

In the preferred embodiment, the mRNA is analyzed by quantitative PCR (to determine the efficiency of splicing) and protein expression is measured using ELISA as described in Example 1 herein.

The product of interest preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal. When the product gene is expressed in a recombinant cell other than one of human origin, the product of interest is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the product of interest from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the product of interest. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The product of interest thereafter is purified from contaminant soluble proteins and polypeptides, for example, by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel electrophoresis using, for example, Sephadex G-75; chromatography on plasminogen columns to bind the product of interest and protein A Sepharose columns to remove contaminants such as IgG.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated by reference.

EXAMPLE 1

tPA production using the dicistronic expression vectors

It was sought to increase the level of homogeneity with regard to expression levels of stable clones by expressing a selectable marker (such as DHFR) and the protein of interest from a single promoter. These vectors divert most of the transcript to product expression while linking it at a fixed ratio to DHFR expression via differential splicing.

Vectors were constructed which were derived from the vector pRK (Suva *et al.*, Science, 237:893-896 [1987]) which contains an intron between the cytomegalovirus immediate early promoter (CMV) and the cDNA that encodes

the polypeptide of interest. The intron of pRK is 139 nucleotides in length, has a splice donor site derived from cytomegalovirus immediate early gene (CMVIE), and a splice acceptor site from an IgG heavy chain variable region (V_H) gene (Eaton *et al.*, Biochem., 25:8343 [1986]).

DHFR/intron vectors were constructed by inserting an EcoRV linker into the BSTX1 site present in the intron of pRK7. An 830 base-pair fragment containing a mouse DHFR coding fragment was inserted to obtain DHFR intron expression vectors which differ only in the sequence that comprises the splice donor site. Those sequences were altered by overlapping PCR mutagenesis to obtain sequences that match splice donor sites found between exons 3 and 4 of normal and mutant Ras genes. PCR was also used to destroy the splice donor site.

A mouse DHFR cDNA fragment (Simonsen *et al.*, Proc. Natl. Acad. Sci. USA, 80:2495-2499 [1983]) was inserted into the intron of this vector 59 nucleotides downstream of the splice donor site. The splice donor site of this vector was altered by mutagenesis to change the ratio of spliced to non-spliced message in transfected cells. It has previously been shown that a single nucleotide change (G to A) converted a relatively efficient splice donor site found in the normal ras gene into an inefficient splice site (Cohen *et al.*, Nature, 334:119-124 [1988]). This effect has been demonstrated in the context of the ras gene and confirmed when these sequences were transferred to human growth hormone constructs (Cohen *et al.*, Cell, 58:461-472 [1989]). Additionally, a non functional 5' splice site (GT to CA) was constructed as a control (ΔGT). A polylinker was inserted 35 nucleotides downstream of the 3' splice site to accept the cDNA of interest. A vector containing tPA (Pennica *et al.*, Nature, 301:214-221 [1983]) was linearized downstream of the polyadenylation site before it was introduced into CHO cells (Potter *et al.*, Proc. Natl. Acad. Sci. USA, 81:7161 [1984]).

Plasmid DNA's that contained DHFR/intron, tPA and (a) wild type ras (WT ras), *i.e.* Figure 3 (SEQ ID NO: 1), (b) mutant ras, or (c) non-functional splice donor site (ΔGT) were introduced into CHO DHFR minus cells by electroporation. The intron vectors were each linearized downstream of the polyadenylation site by restriction endonuclease treatment. The control vector was linearized downstream of the second polyadenylation site. The DNA's were ethanol precipitated after phenol/chloroform extraction and were resuspended in 20μl 1/10 Tris EDTA. Then, 10μg of DNA was incubated with 10^7 CHO.dp12 cells (EP 307,247 published 15 March 1989) in 1 ml of PBS on ice for 10 min. before electroporation at 400 volts and 330μf using a BRL Cell Porator.

Cells were returned to ice for 10 min. before being plated into non-selective medium. After 24 hours cells were fed nucleoside-free medium to select for stable DHFR+ clones which were pooled. The pooled DHFR+ clones were lysed and mRNA's were prepared.

To prepare the mRNA, RNA was extracted from 5 x 10^7 cells which were grown from pools of more than 200 clones derived from the stable

transfection of the three vectors, the essential construction of which is shown in Figure 1B and from non-transfected CHO cells. RNA was purified over oligo-DT cellulase (Collaborative Biomedical Products). 10μg of mRNA was then subjected to Northern blotting which involved running the mRNA on a 1.2% agarose, 6.6% formaldehyde gel, and transferring it to a nylon filter (Stratagene Duralon-UV membrane), prehybridized, probed and washed according to the manufacturer's instructions.

The filter was probed sequentially using probes (shown in Figure 1B) that would detect (a) the full length message, (b) both full length and spliced message, or (c) beta actin. Probing with the long probe showed that the vector that contains the efficient splice donor site (*i.e.* WT ras) generates predominately a mRNA of the size predicted for the spliced product while the other two vectors gave rise primarily to a mRNA that corresponds in size to non-spliced message. The DHFR probe detected only full length message and demonstrated that the WT ras splice donor derived vector generates very little full length message with which to confer a DHFR positive phenotype.

Figure 4 shows the number of DHFR positive colonies obtained after duplicate electroporations with the three intron vectors described above and from a conventional vector that has a CMV promoter driving tPA and a SV40 promoter driving DHFR (see Figure 2). The increase in colony number parallels the increase in full length message that accumulates with the modification of the splice donor sites. The conventional vector efficiently generates colonies and does not vary significantly from the ΔGT construct.

The level of tPA expression was determined by seeding cells in 1 ml of F12:DMEM (50:50, with 5% FBS) in 24 well dishes to near confluency. Growth of the cells continued until the media was exhausted. Media was then assayed by ELISA for tPA production. Briefly, anti-tPA antibody was coated onto the wells of an ELISA microtiter plate, media samples were added to the wells followed by washing. Binding of the antigen (tPA) was then quantified using horse radish peroxidase (HRPO) labelled anti-tPA antibody.

Figure 5A depicts the titers of secreted tPA protein after pooling the clones of each group shown in Figure 4. While the number of colonies increased with a weakening of splice donor function, the inverse was seen with respect to tPA expression. The expression levels are consistent with the RNA products that are observed; as more of the dicistronic message is spliced an increased amount of message will contain tPA as the first open reading frame resulting in increased tPA expression. A mutation of GT to CA in the splice donor site results in an abundance of DHFR positive colonies which express undetectable levels of tPA, possibly resulting from inefficient utilization of the second AUG. Importantly, Figure 5A also shows that expression levels obtained from one of the dicistronic vectors (with WT ras SD) was about threefold higher than that obtained with the control vector containing a CMV promoter/enhancer driving tPA, SV40

promoter/enhancer controlling DHFR and SV40 polyadenylation signals controlling the expression of tPA and DHFR.

Additionally, the homogeneity of expression in the pools was investigated. Figure 5B shows that all 20 clones generated by the WT ras splice donor site derived dicistronic vectors express detectable levels of tPA while only 4 of 20 clones generated by the control vector express tPA. None of the clones transfected with the non-splicing (ΔGT) vector expressed tPA levels detectable by ELISA. This finding is consistent with previous observations that relatively few clones generated by conventional vectors make useful levels of protein.

Expression of tPA was increased following methotrexate amplification of pools. Figure 5C shows that 2 of the dicistronic vector derived pools (*i.e.* with WT ras and MUTANT ras SD sites) increased in expression markedly (8.4 and 7.7 fold), while the pool generated by the conventional vector increased only slightly (2.8 fold) when each was subjected to 200 nM Mtx. An overall increase of 9 fold was obtained using the best dicistronic (WT ras SD) versus the conventional vector following amplification. Growth of the highest expressing amplified pool in nutrient rich production medium yielded titers of 4.2 μg/ml tPA.

It was shown that manipulation of the splice donor sequence alters the ratio of spliced to full length message and the number of colonies that form in selective medium. It was also shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Surprisingly, it was possible to isolate high expressors which had the efficient WT ras splice donor site by selection for DHFR$^-$ cells despite the efficiency with which the DHFR gene was spliced from the RNA precursors formed in these cells.

EXAMPLE 2

TNFr-IgG production using the dicistronic expression vectors

To prove the general applicability of this approach, a second product was evaluated in the dicistronic vector system containing, as the DNA of interest, an immunoadhesin (TNFr-IgG) capable of binding tumor necrosis factor (TNF) (Ashkenazi *et al.*, Proc. Natl. Acad. Sci. USA, 88:10535-10539 [1991]). The experiments described in Example 1 above were essentially repeated except that the product gene encoded the immunoadhesin TNFr-IgG. Plasmid DNA's that contained a TNFr-IgG cDNA and (a) WT ras, *i.e.* Figure 6 (SEQ ID NO: 2), (b) mutant ras or (c) nonfunctional splice donor site (ΔGT) were introduced into the dp12.CHO cells as discussed for Example 1. See Figure 1C for an illustration of the DNA constructs.

It was discovered that the number of DHFR positive colonies generated by three of these vectors was similar to that seen with the tPA constructs. Expression of TNFr-IgG also paralleled that seen with the tPA constructs (Figure 7A). Amplification of pools from two of the constructs showed a marked increase in expression of immunoadhesin (9.6 and 6.8 fold) (Figure

7B). The best of these amplified pools expressed 9.5 μg/ml when grown in nutrient rich production medium.

Thus, it was again shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Furthermore, contrary to expectations, it was discovered that isolation of high product expressing host DHFR$^-$ cells was possible using an efficient splice donor site (*i.e.* the WT ras splice donor site).

EXAMPLE 3

Antibody production using a dicistronic expression vector

The usefulness of this system for antibody expression was evaluated by testing production of an antibody directed against IgE (Presta *et al.*, Journal of Immunology, 151:2623-2632 [1993]). Further, the flexibility of the system with regard to transcription initiation was tested by replacing the CMV promoter/enhancer present in the previous vectors with the promoter/ enhancer derived from the early region of SV40 virus (Griffin, B., Structure and Genomic Organization of SV40 and Polyoma Virus, In J. Tooze [Ed] DNA Tumor Viruses, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The heavy chain of the antibody was inserted downstream of DHFR as described in the earlier tPA and TNFr-IgG constructs. Additionally, a new splice donor site sequence (GAC:GTAAGT) was engineered into the vector which matches the consensus splice donor site more closely than did the splice donor sites present in the vectors tested in Examples 1 and 2. The resultant expression vector is shown in Figures 1D and 9.

It was discovered that this vector produced fewer colonies than the vectors previously tested, and produced predominantly a spliced RNA product. A second vector was constructed to have the light chain of the antibody under control of the SV40 promoter/enhancer and poly-A and the hygromycin B resistance gene under control of the CMV promoter/enhancer and SV40 poly-A. These vectors were linearized at unique HpaI sites downstream of the poly-A signal, mixed at a ratio of light chain vector to heavy chain vector of 10:3 and electroporated into CHO cells using an optimized protocol (as discussed in Examples 1 and 2).

Figure 11 shows the levels of antibody expressed by clones and pools after selection in hygromycin B followed by selection for DHFR expression. All 20 of the clones analyzed expressed high levels of antibody when grown in rich medium and varied from one another by only a factor of four. A pool of antibody producing clones was generated and assayed shortly after it was established. That pool was grown continuously for 6 weeks without a significant decrease in productivity demonstrating that its stability was sufficient to generate gram quantities of protein from its large scale culture.

The pool was subjected to methotrexate amplification at 200nM and 1μM and achieved a greater than 2 fold increase in antibody titer. The 1μM Mtx resistant pool achieved a titer of 41 mg/L when grown under optimal conditions in suspension culture.

The structure of the expressed antibody was examined. Proteins expressed by the 200nM methotrexate resistant pool and by a well characterized expression clone generated by conventional vectors (Presta *et al.* [1993], *supra*) were metabolically labeled with S^{35} cysteine and methionine. In particular, confluent 35mm plates of cells were metabolically labeled with 50μCi each S-35 methionine and S-35 cysteine (Amersham) in serum free cysteine and methionine free F12:DMEM. After one hour, nutrient rich production media was added and labeled proteins were allowed to "chase" into the medium for six more hours. Proteins were run on a 12% SDS/PAGE gel (NOVEX) non-reduced or following reduction with B-mercaptoethanol. Dried gels were exposed to film for 16 hours. CHO control cells were also labeled.

The majority of the antibody protein is secreted with a molecular weight of about 155 kilodaltons, consistent with a properly disulfide-linked antibody molecule with 2 light and 2 heavy chains. Upon reduction the molecular weight shifts to 2 approximately equally abundant proteins of 22.5 and 55 kilodaltons. The protein generated from the pool is indistinguishable from the antibody produced by the well characterized expression clone, with no apparent increase of free heavy or light chain expressed by the pool.

CONCLUSION

The efficient expression system described herein utilizes vectors consisting of promoter/enhancer elements followed by an intron containing the selectable marker coding sequence, followed by the cDNA of interest and a polyadenylation signal.

Several splice donor site sequences were tested for their effect on colony number and expression of the cDNA of interest. A non-functional splice donor site, splice donor sites found in an intron between exons 3 and 4 of mutant (mutant ras) and normal (WT ras) forms of the Harvey Ras gene and another efficient SD site (see Example 3) were used. The vectors were designed to direct expression of dicistronic primary transcripts. Within a transfected cell some of the transcripts remain full length while the remainder are spliced to excise the DHFR coding sequence. When the splice donor site is weakened or destroyed an increase in colony number is observed.

Expression levels show the inverse pattern, with the most efficient splice donor sites generating the highest levels of tPA, TNFr immunoadhesin or anti-IgE V_H.

The homogeneity of expression of clones generated by the ras splice donor site intron DHFR vectors was compared to clones generated from a conventional vector with a separate promoter/enhancer and polyadenylation signal for each DHFR and tPA. The DHFR intron vector gives rise to colonies that are much more homogeneous with regard to expression than those generated by the conventional vector. Non-expressing clones derived from the conventional vector may be the result of breaks in the tPA or

TNFr-IgG domain of the plasmid during integration into the genome or the result of methylation of promoter elements (Busslinger *et al.*, Cell, 34:197-206 [1983]; Watt *et al.*, Genes and Development, 2:1136-1143 [1988]) driving tPA or TNFr-IgG expression. Promoter silencing by methylation or breaks in the DHFR-intron vectors would very likely render them incapable of conferring a DHFR positive phenotype.

It was found that pools generated by the DHFR-intron vectors could be amplified in methotrexate and would increase in expression by a factor of 8.4 (tPA), or 9.8 (TNFr-IgG). Pools from conventional vectors increased by only 2.8 and 3.0 fold for tPA and TNFr-IgG when amplified similarly. Amplified pools resulted in 9 fold higher tPA levels and 15 fold higher TNFr-IgG levels when compared to the conventional vector amplified pools.

Without being limited to any theory, the increase in expression of methotrexate resistant pools derived from the dicistronic vectors is likely due to the transcriptional linkage of DHFR and the product; when cells are selected for increased DHFR expression they consistently over-express product. Conventional approaches lack selectable marker and cDNA expression linkage and therefore methotrexate amplification often generates DHFR overexpression without the concomitant increase in product expression.

A further increase of 4 and 6.3 fold in expression were obtained when amplified tPA and TNFr-IgG pools were transferred from the media used for the selections and amplifications to a nutrient rich production medium.

In Example 3, the expression vector had a splice donor site that more closely matches the consensus splice donor sequence and had the heavy chain of a humanized anti-IgE antibody inserted downstream. This vector was linearized and co-electroporated with a second linearized vector that expresses the hygromycin resistance gene and the light chain of the antibody each under the control of its own promoter/enhancer and poly-A signals. An excess of light chain expression vector over the heavy chain dicistronic expression vector was used to bias in favor of light chain expression. Clones and a pool were generated after hygromycin B and DHFR selections. The clones were found to express relatively consistent, high levels of antibody, as did the pool. The 1μM pool achieved a titer of 41mg/L when grown under optimal conditions in suspension culture.

The anti-IgE antibody was assessed by metabolic labeling followed by SDS/PAGE under reducing and non reducing conditions and found to be indistinguishable from the protein expressed by a highly characterized clonal cell line. Of particular importance is the finding that no free light chain is observed in the pool relative to the clone.

A stable expression system for CHO cells has been developed that produces high levels of recombinant proteins rapidly and with less effort than that required by other expression systems. The vector system generates stable clones that express consistently high levels thereby reducing the number of clones that must be screened to obtain a highly productive clonal line. Alternatively, pools have been used to conveniently generate moderate to high levels of protein. This approach

may be particularly useful when a number of related proteins are to be expressed and compared.

Without being limited to this theory, it is possible the vectors that have very efficient splice donor sites generate very productive clones because so little transcript remains non spliced that only integration events that lead to the generation of high levels of RNA produce enough DHFR protein to give rise to colonies in selective medium. The high level of spliced message from such clones is then translated into abundant amounts of the protein of interest. Pools of clones made concurrently by introducing conventional vectors expressed lower levels of protein, and were unstable with regard to long term expression, and expression could not be appreciably increased when the cells were subjected to methotrexate amplification.

The system developed herein is versatile in that it allows high levels of single and multiple subunit polypeptides to be rapidly generated from clones or pools of stable transfectants. This expression system combines the advantages of transient expression systems (rapid and labor non intensive generation of research amounts of protein) with the concurrent development of highly productive stable production cell lines.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GENENTECH, INC.

(ii) TITLE OF INVENTION: METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/286740
(B) FILING DATE: 05-AUG-1994

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7360 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA 750

GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT 800

GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA 850

CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC 900

AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA 950

CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT 1000

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT 1600

AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG 1650

TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC 1700

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GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC 1850

AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC 1900

GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG 1950

CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT 2000

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CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG 2650

GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA 2700

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GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT 2800

GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT 2850

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TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG 3450

GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT 3500

GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA 3550

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GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA 3650

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TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA 3750

TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA 3800

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TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC 5500

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GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT 5600

ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC 5650

ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC 5700

ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC 5750

GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC 5800

TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT 5850

GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC 5900

TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG 5950

AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC 6000

TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT 6050

CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT 6100

ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT 6150

GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA 6200

CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA 6250

TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT 6300

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TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA 6400

AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT 6450

CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT 6500

CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC 6550

CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT 6600

GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA 6650

TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT 6700

TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA 6750

GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG 6800

CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG 6850

CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT 6900

CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG 6950

CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA 7000

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TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA 7250

TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG 7300

GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT 7350

TACGAATTAA 7360

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6889 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

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AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

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GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC 1600

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TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG 2900

CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG 2950

CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC 3000

AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC 3050

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GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT 3150

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TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA 3250

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GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC 3350

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ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT 4250

TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG 4300

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TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT 4900

TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT 4950

GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA 5000

CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA 5050

TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC 5100

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AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA 6800

CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT 6850

TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA 6889

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6557 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG 50

GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA 100

GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG 150

TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA 200

GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC 250

CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT 300

TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG 350

AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG 400

CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA 450

GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT 500

TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA 550

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TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA 700

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GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA 800

ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA 850

GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT 900

GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT 950

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TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC 2000

CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT 2050

GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC 2100

TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT 2150

CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA 2200

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CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG 2500

ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG 2550

ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA 2600

GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT 2650

CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC 2700

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TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC 4300

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AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG 6000

CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT 6050

GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA 6100

TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA 6150

CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT 6200

TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT 6250

GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC 6300

AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG 6350

CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG 6400

AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA 6450

GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA 6500

TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC 6550

GAATTAA 6557

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7305 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA 750

GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA 800

CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA 850

CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC 900

ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG 950

TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT 1000

GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG 1050

GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA 1100

GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA 1150

AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC 1200

GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC 1250

GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA 1300

TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC 1350

AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG 1400

TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA 1450

GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC 1500

ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC 1550

CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA 1600

ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG 1650

AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG 1700

CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG 1750

CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG 1800

ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC 1850

CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA 1900

TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA 1950

GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC 2000

CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA 2050

CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG 2100

TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT 2150

TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA 2200

GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG 2250

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ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA 2350

GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC 2400

GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT 2450

CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC 2500

GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG 2550

CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG 2600

AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT 2650

ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA 2700

ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC 2750

CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC 2800

TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT 2850

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GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT 2950

GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG 3000

TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC 3050

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CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC 3250

CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG 3300

CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA 3350

CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA 3400

AGGACAGCAC CTACAGCCTC AGCAGCACCC TGACGCTGAG CAAAGCAGAC 3450

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CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG 3750

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GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG 4650

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CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA 6750

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TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG 6900

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CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA 7150

AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG 7200

CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT 7250

GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA 7300

ATTAA 7305

CLAIMS

1. A DNA construct comprising a transcriptional initiation site, a transcriptional termination site, a selectable gene, a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene being positioned within an intron having a splice donor site 5' of the intron, which splice donor site regulates expression of the product gene using the transcriptional regulatory region.

2. The DNA construct of claim 1 wherein the splice donor site comprises an efficient splice donor sequence.

3. The DNA construct of claim 2 wherein the splice donor site comprises a consensus splice donor sequence.

4. The DNA construct of claim 2 wherein the splice donor site comprises the sequence GACGTAAGT.

5. The DNA construct of claim 1 wherein the selectable gene is an amplifiable gene.

6. The DNA construct of claim 5 wherein the amplifiable gene is DHFR.

7. The DNA construct of claim 1 wherein the transcriptional regulatory region comprises a promoter and an enhancer.

8. A vector comprising the DNA construct of claim 1.

9. The vector of claim 8 wherein the selectable gene of the DNA construct is an amplifiable gene.

10. The vector of claim 8 that is capable of replication in a eukaryotic host.

11. A eukaryotic host cell comprising the vector of claim 10.

12. A eukaryotic host cell comprising the DNA construct of claim 5.

13. The host cell of claim 11 wherein the vector is introduced into the host cell by electroporation.

14. A eukaryotic host cell comprising the DNA construct of claim 1 integrated into a chromosome of the host cell.

15. The host cell of claim 14 that is a mammalian cell.

16. A method for producing a product of interest comprising culturing the host cell of claim 11 so as to express the product gene and recovering the product from the host cell culture.

17. The method of claim 16 further comprising recovering the product from the culture medium.

18. The method of claim 16 wherein the selectable gene is an amplifiable gene and the splice donor site comprises an efficient splice donor sequence.

19. A method for producing a product of interest comprising culturing the host cell of claim 12 so as to express the product gene in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, and recovering the product.

20. A method for producing eukaryotic cells having multiple copies of a product gene comprising transforming eukaryotic cells with the DNA construct of claim 5, growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene.

21. The method of claim 20 further comprising recovering from the selected cells the product of interest.

FIG. 1A

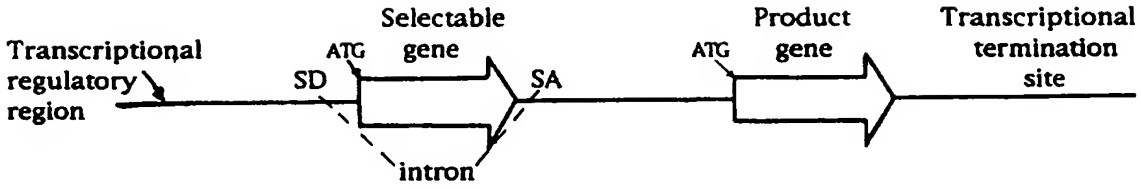
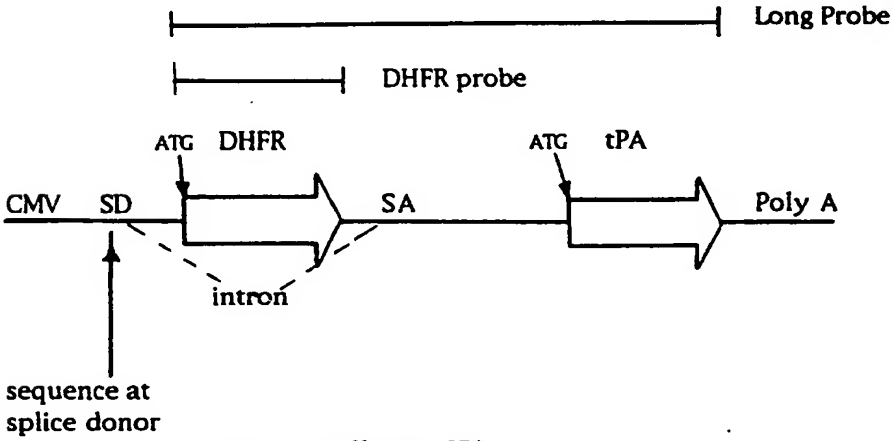
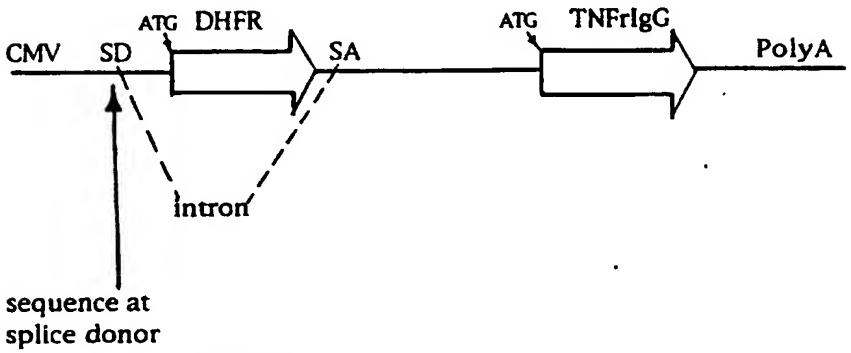


FIG. 1B



a) WT ras (efficient SD)
b) MUTANT ras (less efficient SD)
c) ▲GT (inefficient SD)

FIG. 1C



a) WT ras
b) MUTANT ras
c) ▲GT

FIG. 1D

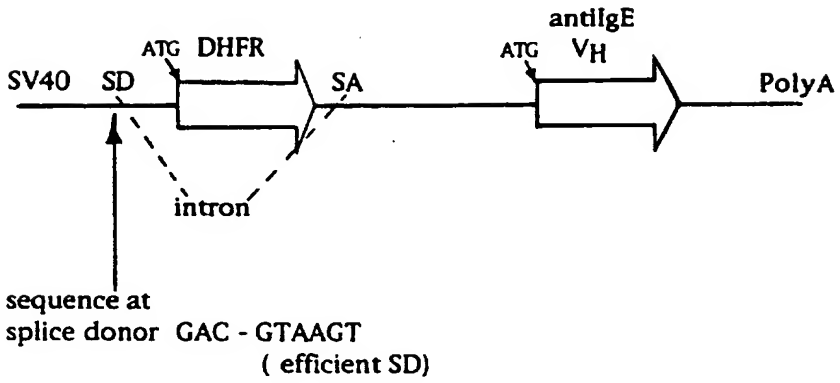

antiIgE
ATG DHFR
ATG V_H
SV40 SD
SA
PolyA
intron
sequence at
splice donor GAC - GTAAGT
(efficient SD)

FIG. 2

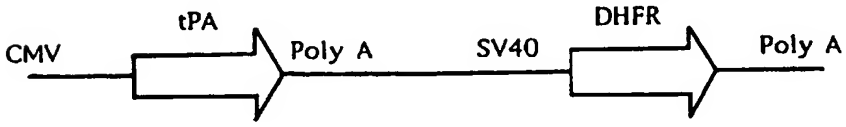

tPA
DHFR
CMV
Poly A
SV40
Poly A

FIG. 3A

```
        aluI
       sstI
       sacI
       hgiJII
       hgiAI/aspHI
       ecl136II
       bsp1286                                                                                     thaI
       bsiHKAI                                                                                     fnuDII/mvnI
       bmyI                       rmaI    tru9I                                                    bstUI
       banII                      maeI    mseI                                                     bsh1236I
     taqI                        speI   aseI/asnI/vspI                           bslI            aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                      scrFI
                      mvaI
                      ecoRII
                      dsaV
                   aciI
                 bglI bstNI                                maeII
                sau96I                                   hinlI/acyI
                haeIII/palI     aciI                     ahaII/bsaHI
                asuI  apyI[dcm+]                aciI     aatII          maeII           maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

       maeII                                                                                      maeII
      hinlI/acyI                                                                                 hinlI/acyI
      ahaII/bsaHI                         rsaI                        rsaI                       ahaII/bsaHI
      aatII                         bglI         csp6I            ndeI       csp6I          aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII
           aciI                                                                                      nlaIII
         bglI  dsaV                                                                           styI
        sau96I bstNI            rsaI                                         maeII                  ncoI
        haeIII/palI             csp6I                              rsaI     snaBI                   dsaI  hphI  aciI
        asuI   apyI[dcm+]   bsrI  nlaIII                          csp6I    bsaAI                   bsaJI  sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 3B

```
                                                                                             maeII
                                                                                           hinlI/acyI                nlaIV
           rsaI                                      pleI                                  ahaII/bsaHI               hgiCI
           csp6I                           aciI      hinfI                    bsmAI        aatII                     banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                              aluI
                                                                                                             sstI
                                                                                                             sacI
                                                                                                             hgiJII
                                                                                                             hgiAI/aspHI
                                                                                                             ecl136II
                                                                                                             bsp1286
                                                                                                             bsiHKAI
                                                                                   rsaI                      bmyI
                                  maeIII    aciI       hgaI         aciI          csp6I      mnlI            banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                    haeIII/palI
                                                                                                   mcrI
                                                                                                   eagI/xmaIII/eclXI
                                                                                                   eaeI
                                                                                                   cfrI
                                                                                                  fnu4HI
                                                                                                  aciI
                                                                                                 thaI
                       esp3I                                                                     fnuDII/mvnI
                  scrFI                                                    sau96I            sacII/sstII
                  mvaI bsmAI                                                     avaII             nspBII
                  ecoRII                                                   asuI              kspI  scrFI
                  dsaV                                                    nlaIV              dsaI  nciI
                  bstNI  hinlI/acyI                                           scrFI           bglI bslI  mspI
                  apyI[dcm+]                                                  nciI       sau3AI mnlI bstUI
             sau3AI gsuI/bpmI                                                 mspI       mboI/ndeII[dam-] hpaII
             mboI/ndeII[dam-]                                       mboII     hpaII      dpnI[dam+] bsaJI dsaV
             dpnI[dam+]  hgaI   fokI                                bpuAI     dsaV       dpnII[dam-] bsh1236I
             dpnII[dam-] ahaII/bsaHI                    mnlI        bbsI      cauII      alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 3C

```
                tfiI
            aciI
           thaI hinfI                                                               fnu4HI
           fnuDII/mvnI                         aciI                                 bbvI
           bstUI                            rsaI                                  nspBII           taqI
           bsh1236I                         csp6I   scfI           mnlI           aciI       nlaIII
 701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                 thaI
                                                                                 fnuDII/mvnI
                                                                                 bstUI
                                                                                 bsh1236I
                                                                               mluI
                      pflMI                        bsmAI             bsrBI     aflIII     rsaI
     sfaNI            bslI                         bsaI              aciI    xmnI         csp6I
                                                                  mnlI   ddeI asp700     scaI
 801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
     CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                            scrFI
                                                            mvaI
                                                            ecoRII
          eco57I                                            dsaV
         mboII                   tfiI                       bstNI                  tfiI      tru9I         tru9I
        earI/ksp632I             hinfI                      apyI[dcm+]         mboII  taqI   mseI          mseI
       mnlI                    alwNI    hphI              sexAI           ddeI    hinfI     ahaIII/draI   aseI/asnI/vspI
 901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
     TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                              aluI
                                             sstI
                                             sacI
                                             hgiJII
                                             hgiAI/aspHI
                                             ecl136II
                                             bsp1286
                                             bsiHKAI
                                             bmyI                                   tru9I              mspI
                                             banII                     sfaNI mseI                hpaII
           ddeI                   bslI     mnlI                bstXI      fokI       aflII/bfrI        bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 3D

```
                                                                                         haeIII/palI
                                                                                        haeI
                                                               scrFI          scrFI                              nlaIII
                                                               mvaI                 mvaI                                   sau3AI
                                                               ecoRII         ecoRII                          mboI/ndeII[dam-]
                                                               dsaV    tfiI   dsaV    pleI                    dpnI[dam+]
                                                               bstNI      nlaIII   bstNI      ddeI                        dpnII[dam-]
               accI  nlaIII                  mnlI              apyI[dcm+]    hinfI   apyI[dcm+]     hinfI      maeIII    alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                               mnlI
                                                                                          hinlI/acyI         scrFI
                                                                                          ahaII/bsaHI        mvaI
                                                                                       scrFI          ecoRII
                                                                                       mvaI    ecoNI         dsaV
                                                                                       ecoRII      sau96I
                                                                                       dsaV        avaII
                             maeII                                                     bstNI   bslI      asuI    mnlI
                           aflIII                                                      apyI[dcm+]      mnlI  bstNI
         apoI           maeIII                                          mnlI        bsaJI hgaI       ddeI    apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                                     ppu10I
                                                                  sfaNI                              nsiI/avaIII
         sfaNI                  accI      mboII                 mboII                     mnlI     aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                                fnu4HI
        nlaIII                                                  aciI
       styI                                                    thaI
       ncoI                                                    fnuDII/mvnI tru9I
       dsaI                                  styI              bstUI       mseI                                 maeIII
       bsaJI                                 bsaJI             bsh1236I  aseI/asnI/vspI                        hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 3E

```
                                                       scrFI
                                                       mvaI
                                                       ecoRII                           bspMI
                                                       dsaV                           scfI
                                                       bstNI                              pstI
                                                       apyI[dcm+]      mnlI        aluI   bsgI               tru9I
                                                     gsuI/bpmI         bsaJI     hindIII fnu4HI              mseI
      scfI        fokI                           maeIII     hincII/hindII     ddeI       bbvI            apoI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATTCGAACCC GACGTCCAGC GGCACTTAAA

                                          hgiJII                                                 scrFI
                                          bsp1286                                                mvaI
                                          bmyI                fnu4HI                             ecoRII
                        sfaNI           mnlI                  bbvI               mboII           dsaV
                       fokI       mboII   banII            fnu4HI               bpuAI            bstNI
        hgaI         nlaIII       earI/ksp632I             bbvI                 bbsI             apyI[dcm+] nlaIII
1601 AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC
     TTCCCTGCGA CACTTCGTTA GTACCTACGT TACTTCTCTC CCGAGACGAC ACACGACGAC GACACACCTC GTCAGAAGCA AAGCGGGTCG GTCCTTTAGG

                                            scfI
                        sau3AI              pstI
                        mboI/ndeII[dam-]    bsgI
                 nlaIV  dpnI[dam+]        sau3AI                                                  hgiAI/aspHI
               mnlI    bstYI/xhoII        mboI/ndeII[dam-]                                   hinPI bsp1286
      tfiI   mboII     bglII              dpnI[dam+]                                      fnu4HI   bsiHKAI
      hinfI  earI/ksp632I dpnII[dam-]     dpnII[dam-]                                    nlaIII   hhaI/cfoI bmyI
1701 ATGCCCGATT CAGAAGAGGA GCCAGATCTT ACCAAGTGAT CTGCAGAGAT GAAAAAACGC AGATGATATA CCAGCAACAT CAGTCATGGC TGCGCCCTGT
     TACGGGCTAA GTCTTCTCCT CGGTCTAGAA TGGTTCACTA GACGTCTCTA CTTTTTTGCG TCTACTATAT GGTCGTTGTA GTCAGTACCG ACGCGGGACA

            scrFI
            nciI
            mspI
            hpaII                                           alwNI
            dsaV                             bsp1286      ddeI                    fnu4HI   styI
      ddeI  cauII      sspI                  bmyI       draIII                   bbvI     bsaJI
1801 GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC
     CGAGTCTTCG TTGGCCCACC TTATAACGAC CACGTTGTCA CCGTCCCGTG TCACGGTGAG TCACGGACAG TTTTCAACGT CGCTCGGTTC CACAAAGTTG
```

FIG. 3F

```
                                                                                                                        pmlI
                                                                                                                        eco72I
                                                                                                                    sau96I
                                                                                                                    asuI maeII
                                                                                                                scrFI
      bspMI                                                                                                     mvaI haeIII/palI
   nlaIV         sau96I                                                                                         ecoRII
   hgiCI         haeIII/palI                                                                                    dsaV
   banI          asuI   rsaI                           bsp1286                                                  bstNI    bsaAI
  bsp1286       eco0109I/draII                         bmyI                                                     bsaJI    bbrPI
  bmyI          alwNI    csp6I ddeI                 bsrI       bcgI                                             apyI[dcm+]
1901 GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT
     CCCCCGTGGA CGGTCGTCCG GGACATGAAG AGTCTAAAGC ACACGGTCAC GGGGCTTCCT AAACGACCCT TCACGACACT TTATCTATGG TCCCGGTGCA

                scrFI                                          hinPI
                mvaI                                                 hhaI/cfoI
                ecoRII                                        nlaIV    hgiAI/aspHI
                dsaV                                          narI     bsp1286                 thaI
                bstNI                      pmlI  hgiAI/aspHI        kasI     bsiHKAI                 fnuDII/mvnI
                bsaJI                      eco72I                   hinlI/acyI                       bstUI sau96I
            sau96I                   bsaAI bsp1286              hgiCI  bmyI                  hinPI   haeIII/palI
            avaII                    bbrPI bsiHKAI              haeII  apaLI/snoI            hhaI/cfoI
            asuI      sfaNI   scfI   bsp1286    bmyI     aciI       banI     alw44I/snoI       fnu4HI     asuI        bslI
          mnlI  apyI[dcm+] aluI     bmyI maeII       nspBII        ahaII/bsaHI      bsrI       bbvI bsh1236I        bslI
2001 GCTACGAGGA CCAGGGCATC AGCTACAGGG GCACGTGGAG CACAGCGGAG AGTGGCGCCG AGTGCACCAA CTGGAACAGC AGCGCGTTGG CCCAGAAGCC
     CGATGCTCCT GGTCCCGTAG TCGATGTCCC CGTGCACCTC GTGTCGCCTC TCACCGCGGC TCACGTGGTT GACCTTGTCG TCGCGCAACC GGGTCTTCGG

                                         scrFI
                                         mvaI
                                         ecoRII
                                         dsaV                                                pleI       scrFI
                                         bstNI                                                  bsmAI        mvaI
                                         bsaJI                                                taqI[dam-]     ecoRII
                           hinlI/acyI    apyI[dcm+]                                          sau3AI hinfI    dsaV
                aciI  haeIII/palI       haeIII/palI                 scfI                     mboI/ndeII[dam-] bstNI
   nspBII          haeI  hgaI bstXI     sau96I              pstI                        dpnI[dam+]      apyI[dcm+]     tru9I
  scfI aciI      mnlI   ahaII/bsaHI     asuI      nlaIV           bsgI                   dpnII[dam-]      bsaJI        maeII mseI
2101 CTACAGCGGG CGGAGGCCAG ACGCCATCAG GCTGGGCCTG GGGAACCACA ACTACTGCAG AAACCCAGAT CGAGACTCAA AGCCCTGGTG CTACGTCTTT
     GATGTCGCCC GCCTCCGGTC TGCGGTAGTC CGACCCGGAC CCCTTGGTGT TGATGACGTC TTTGGGTCTA GCTCTGAGTT TCGGGACCAC GATGCAGAAA
```

FIG. 3G

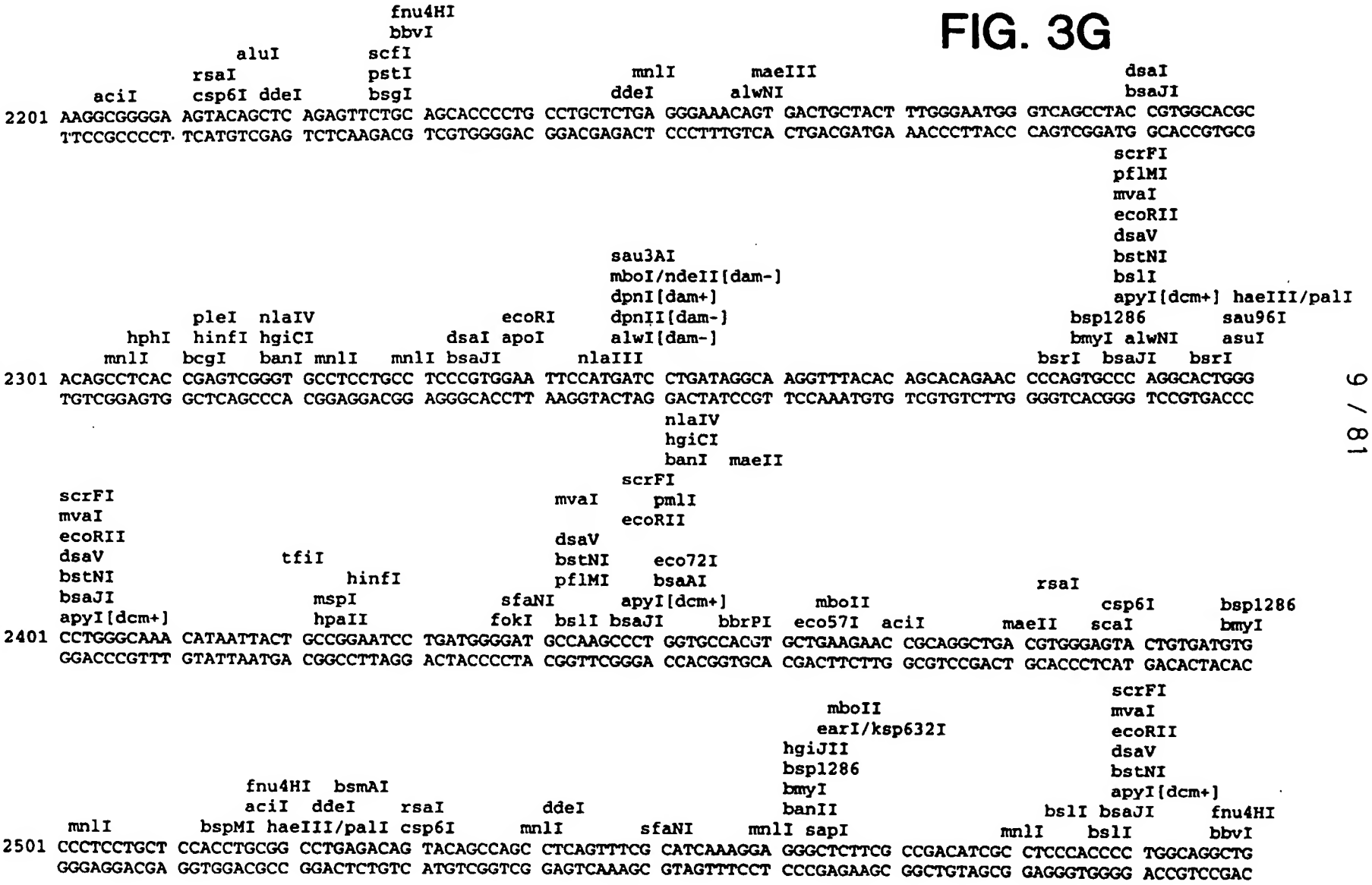


FIG. 3H

```
                                  mspI
                                  hpaII                                                                 scrFI
                                  bslI                                                                  mvaI
                                 scrFI                                                                  ecoRII
                                 nciI       nlaIV                                                       dsaV
                                 dsaV     aciI                          aluI        tfiI        aciI          bstNI
              bslI       mnlI    cauII  bsrBI   bslI aciI             alwNI        hinfI      fnu4HI        apyI[dcm+]
2601 CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA
     GGTAGAAACG GTTCGTGTCC TCCAGCGGGC CTCTCGCCAA GGACACGCCC CCGTATGAGT AGTCGAGGAC GACCTAAGAG AGACGGCGGG TGACGAAGGT

                                                                    scrFI
                                                                    mvaI
                                                                    ecoRII
                                                                    dsaV
                                                                    bstNI
                                                                    apyI[dcm+]
                                                         scrFI     bslI
                                      sau3AI             nciI      bsaJI
                                      mboI/ndeII[dam-]   mspI   sau96I
                                      dpnI[dam+]         hpaII  nlaIV
                                      dpnII[dam-]        dsaV   avaII         mnlI
        mnlI    aciI    bslI      hphI[dam-]             cauII  asuI       mnlI         apoI       taqI
2701 GGAGAGGTTT CCGCCCCACC ACCTGACGGT GATCTTGGGC AGAACATACC GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT
     CCTCTCCAAA GGCGGGGTGG TGGACTGCCA CTAGAACCCG TCTTGTATGG CCCACCAGGG ACCGCTCCTC CTCGTCTTTA AACTTCAGCT TTTTATGTAA

                                                         scfI                               scrFI
                                                         pstI                               mvaI
                                                        fnu4HI                              ecoRII
                                                        bbvI pvuII                          dsaV
                                                    fnu4HI fnu4HI                           bstNI                aciI
                                                     bbvI  bbvI                        bsp1286     fnu4HI  sau96I
              ecoRI                                hinPI bsgI aluI        tfiI     nspBII bsaJI     bbvI    avaII
              apoI taqI                            hhaI/cfoI nspBII       hinfI    aciI bmyI apyI[dcm+]     asuI
2801 GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT GCAGCTGAAA TCGGATTCGT CCCGCTGTGC CCAGGAGAGC AGCGTGGTCC
     CAGGTATTCC TTAAGCTACT ACTGTGAATG CTGTTACTGT AACGCGACGA CGTCGACTTT AGCCTAAGCA GGGCGACACG GGTCCTCTCG TCGCACCAGG
```

FIG. 3I

aluI
sstI
sacI
hgiJII
hgiAI/aspHI
ec1136II
bsp1286
bsiHKAI
bmyI mspI
banII hpaII
haeIII/palI
stuI
haeI
mnlI
nlaIII bsmAI
tru9I hphI
mseI maeIII
nlaIII
nspI
nspHI gsuI/bpmI
fnu4HI
nspBII
aciI
fnu4HI
rsaI fokI
csp6I alwNI bbvI
pvuII
scfI
fnu4HI
pstI
bspMI bbvI
sau96I nspBII
avaII fnu4HI
asuI bbvI mspI
bsrI
bsl1 bsaJI aciI bsgI aluI hpaII
draIII
fnu4HI
aciI
bsrBI eco57I mnlI nlaIII
2901 GCACTGTGTG CCTTCCCCCG GCGGACCTGC AGCTGCCGGA CTGGACGGAG TGTGAGCTCT CCGGCTACGG CAAGCATGAG GCCTTGTCTC CTTTCTATTC
CGTGACACAC GGAAGGGGGC CGCCTGGACG TCGACGGCCT GACCTGCCTC ACACTCGAGA GGCCGATGCC GTTCGTACTC CGGAACAGAG GAAAGATAAG
3001 GGAGCGGCTG AAGGAGGCTC ATGTCAGACT GTACCCATCC AGCCGCTGCA CATCACAACA TTTACTTAAC AGAACAGTCA CCGACAACAT GCTGTGTGCT
CCTCGCCGAC TTCCTCCGAG TACAGTCTGA CATGGGTAGG TCGGCGACGT GTAGTGTTGT AAATGAATTG TCTTGTCAGT GGCTGTTGTA CGACACACGA
sau96I
nlaIV
haeIII/palI
asuI
sau96I
nlaIV scrFI
hgiJII
eco0109I/draII
bsp1286
bsp120I
bmyI mvaI
banII ecoRII
aciI dsaV
fnu4HI bstNI
aciI asuI apyI[dcm+]
bsrBI apaI bsaJI
bsmAI
3101 GGAGACACTC GGAGCGGCGG GCCCCAGGCA AACTTGCACG ACGCCTGCCA GGGCGATTCG GGAGGCCCCC TGGTGTGTCT GAACGATGGC CGCATGACTT
CCTCTGTGAG CCTCGCCGCC CGGGGTCCGT TTGAACGTGC TGCGGACGGT CCCGCTAAGC CCTCCGGGGG ACCACACAGA CTTGCTACCG GCGTACTGAA
scrFI
mvaI
ecoRII
dsaV
bstNI
sau96I
nlaIV
haeIII/palI
tfiI asuI apyI[dcm+]
hinlI/acyI eco0109I/draII
hgaI apyI[dcm+] mnlI bsaJI
ahaII/bsaHI hinfI
aciI
fnu4HI
haeIII/palI
eaeI
cfrI nlaIII

FIG. 3J

```
                                                        scrFI
                                                        nciI
                             scrFI                mspI
                             mvaI                       hpaII
                             ecoRII               dsaV
                             dsaV                 cauII
                             bstNI                     xmaI/pspAI
                             bsaJI                     smaI
                             apyI[dcm+]                scrFI
                        nlaIV                          nciI
                        eco0109I/draII           dsaV
                    aluI sau96I                  cauII     rsaI  styI maeIII           tfiI       nspI
                   pvuII asuI                          bsaJI     csp6I bsaJI       rmaI    hinfI       nspHI
           sfaNI   nspBII  haeIII/palI           fokI  avaI       bsp1407I   bstEII    maeI bsrI      maeIII nlaIII
3201 TGGTGGGCAT CATCAGCTGG GGCCTGGGCT GTGGACAGAA GGATGTCCCG GGTGTGTACA CCAAGGTTAC CAACTACCTA GACTGGATTC GTGACAACAT
     ACCACCCGTA GTAGTCGACC CCGGACCCGA CACCTGTCTT CCTACAGGGC CCACACATGT GGTTCCAATG GTTGATGGAT CTGACCTAAG CACTGTTGTA

                                                             mboII
                scrFI                            aciI earI/ksp632I
                mvaI                               sau3AI mnlI
                ecoRII                       mboI/ndeII[dam-]
                dsaV                         dpnI[dam+]            mboII
                bstNI           mnlI               dpnII[dam-]     eco57I
         bslI   apyI[dcm+]   pleI                  alwI[dam-]     mboII  bpuAI           hinPI
      mcrI maeIII            hinfI                bstYI/xhoII  mboII  bbsI            hhaI/cfoI      scfI
3301 GCGACCGTGA CCAGGAACAC CCGACTCCTC AAAAGCAAAT GAGATCCCGC CTCTTCTTCT TCAGAAGACA CTGCAAAGGC GCAGTGCTTC TCTACAGACT
     CGCTGGCACT GGTCCTTGTG GGCTGAGGAG TTTTCGTTTA CTCTAGGGCG GAGAAGAAGA AGTCTTCTGT GACGTTTCCG CGTCACGAAG AGATGTCTGA

                                        bsmAI               mboII
      gsuI/bpmI        aciI     aciI    bsaI  scfI     mnlI earI/ksp632I                                    tth111I/aspI
3401 TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT
     AGAGGTCTGG GTGGTGTGGC GTCTTCGCCC TGCTCTGGGA TGTCCTCTCC CTTCTCACGT AAAAGGGTCT ATGAAGGGTA AAACCTTCAA AAGTCCTGAA

                                                                                      scrFI
                                                                 scrFI       mvaI
                                                                 mvaI bsmI            ecoRII
                                                                 ecoRII      dsaV
                                                                 dsaV        bstNI
                                      mboII                      bstNI                bsaJI
                                      bpuAI   bsmI      rmaI   gsuI/bpmI    mnlI  bsaJI                ecoRI   taqI
                                      bbsI nlaIII       maeI mnlI apyI[dcm+]  mnlI apyI[dcm+]          apoI   claI/bsp106
3501 GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA GCCTCTCCAG GAATGCCTCC TCCCTGGGCA GAAGTGGGGG GAATTCAATC
     CCAGACTAAA GTCCTATGAG ACAGTCTACC CTTCTGTACT TACGTGTGAT CGGAGAGGTC CTTACGGAGG AGGGACCCGT CTTCACCCCC CTTAAGTTAG
```

FIG. 3K

```
                styI
              aciI
             fnu4HI   sau96I
             bglI nlaIII
            sfiI ncoI haeIII/palI
            haeIII/palI                    aluI
           eaeI  dsaI asuI               fnu4HI
           cfrI  bsaJI                   bbvI          maeIII              sfaNI  apoI                                  bsmI
3601 GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA
     CTACCGGCGG TACCGGGTTG AACAAATAAC GTCGAATATT ACCAATGTTT ATTTCGTTAT CGTAGTGTTT AAAGTGTTTA TTTCGTAAAA AAAGTGACGT

                                                          sau3AI
                                                          mboI/ndeII[dam-]
                                                          dpnI[dam+]
                                                          dpnII[dam-]
                                                         pvuI/bspCI
                                                         mcrI
                                                        taqI[dam-]  tru9I
                                                      claI/bsp106[dam-]              styI
                                                     sau3AI          mseI       fnu4HI    haeI
                                                     mboI/ndeII[dam-]           bbvI  ncoI
                                                     dpnI[dam+] xmnI        hinPI    dsaI haeIII/palI
          rmaI                                       dpnII[dam-]    aseI/asnI/vspI    bsaJI
          maeI                                  nlaIII alwI[dam-]  asp700      hhaI/cfoI nlaIII           mnlI
3701 TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA
     AAGATCAACA CCAAACAGGT TTGAGTAGTT ACATAGAATA GTACAGACCT AGCTAGCCCT TAATTAAGCC GCGTCGTGGT ACCGGACTTT ATTGGAGACT

                       rsaI                                                                    nlaIV
                       csp6I                                                                 scrFI
                      nlaIV                                                                  mvaI
                      kpnI                                                                   ecoRII
                      hgiCI                                                                  dsaV
                      banI                              aluI                                 bstNI
                      asp718     mnlI                   pvuII                                apyI[dcm+]
          mnlI        acc65I   ddeI aciI                nspBII                             bsaJI
3801 AAGAGGAACT TGGTTAGGTA CCTTCTGAGG CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA
     TTCTCCTTGA ACCAATCCAT GGAAGACTCC GCCTTTCTTG GTCGACACCT TACACACAGT CAATCCCACA CCTTTCAGGG GTCCGAGGGG TCGTCCGTCT
```

FIG. 3L

```
                                                                  nlaIV
           sfaNI                   scrFI        scrFI                                   sfaNI
         ppu10I                    mvaI               mvaI                                        ppu10I
         nsiI/avaIII               ecoRII       ecoRII                                nsiI/avaIII
        nlaIII                     dsaV         dsaV                                 nlaIII
       sphI                        bstNI              bstNI                                      sphI
       nspI                        apyI[dcm+]         apyI[dcm+]                                 nspI
       nspHI                      sexAI              bsaJI                                       nspHI
3901 AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT
     TCATACGTTT CGTACGTAGA GTTAATCAGT CGTTGGTCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT CTTCATACGT TTCGTACGTA GAGTTAATCA

                                                                                        nlaIII
                                                                                       styI
                                                                                       ncoI
                                     aciI                                        bslI  dsaI
           aciI        aciI   fokI              aciI bsrI  aciI                  aciI  bsaJI
4001 CAGCAACCAT AGTCCCGCCC CTAACTCCGC CCATCCCGCC CCTAACTCCG CCCAGTTCCG CCCATTCTCC GCCCCATGGC TGACTAATTT TTTTTATTTA
     GTCGTTGGTA TCAGGGCGGG GATTGAGGCG GGTAGGGCGG GGATTGAGGC GGGTCAAGGC GGGTAAGAGG CGGGGTACCG ACTGATTAAA AAAAATAAAT

                                                                            rmaI
                                                                           styI
           aciI                                                            bsaJI
          fnu4HI                                                           blnI
         sfiI                                                              avrII
         haeIII/palI                                                     haeIII/palI          tru9I
     bsaJI bglI       ddeI                                              stuI                 .hpaI
   haeIII/palI mnlI haeIII/palI                      mnlI               haeI.                 hincII/hindII
  mnlI   mnlI   bsaJI  mnlI  aluI                mnlI                   mnlI maeI          aluI mseI  aluI
4101 TGCAGAGGCC GAGGCCGCCT CGGCCTCTGA GCTATTCCAG AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTGTT AACAGCTTGG
     ACGTCTCCGG CTCCGGCGGA GCCGGAGACT CGATAAGGTC TTCATCACTC CTCCGAAAAA ACCTCCGGAT CCGAAAACGT TTTTCGACAA TTGTCGAACC

                                        scrFI
                                        mvaI
                                        ecoRII
  haeIII/palI                           dsaV
  eaeI                                  bstNI                                                     aluI
  cfrI                     bsrI         apyI[dcm+]      tru9I       fnu4HI                       pvuII
 bsrI              maeII maeIII        bsaJI   maeIII   mseI        bbvI   fokI                  nspBII
4201 CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCC TTCGCCAGCT GGCGTAATAG
     GTGACCGGCA GCAAAATGTT GCAGCACTGA CCCTTTTGGG ACCGCAATGG GTTGAATTAG CGGAACGTCG TGTAGGGGGG AAGCGGTCGA CCGCATTATC
```

FIG. 3M

```
                                                                  hinPI
                                                                   hhaI/cfoI
                                                                  nlaIV
            sau3AI                                                narI
  sau96I    mboI/ndeII[dam-]                                      kasI
  haeIII/palI                                                     hinlI/acyI
  asuI      dpnI[dam+]                                            hgiCI
 mnlI       dpnII[dam-]                                           haeII     aciI
mboII  aciI pvuI/bspCI                                            banI   sfaNI
earI/ksp632I mcrI                             bglI                ahaII/bsaHI                    sfaNI    aciI
4301 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGT AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT
     GCTTCTCCGG GCGTGGCTAG CGGGAAGGGT TGTCAACGCA TCGGACTTAC CGCTTACCGC GGACTACGCC ATAAAAGAGG AATGCGTAGA CACGCCATAA

                                                                    aciI
                                                                  fnu4HI                fnu4HI
                                  hinPI                           thaI                 hinPI
                                 thaI                             fnuDII/mvnI          hhaI/cfoI
                                 fnuDII/mvnI                      bstUI                thaI
                                 bstUI scfI     hinPI            hinPI                 fnuDII/mvnI
                                 bsh1236I       hhaI/cfoI hhaI/cfoI                    bstUI
                              rsaI hhaI/cfoI  fnu4HI   tru9I aciI                      bsh1236I    aciI
    aciI   maeII              csp6I   bslI    aciI     mseI bsh1236I               maeIII bbvI  maeIII
4401 TCACACCGCA TACGTCAAAG CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC
     AGTGTGGCGT ATGCAGTTTC GTTGGTATCA TGCGCGGGAC ATCGCCGCGT AATTCGCGCC GCCCACACCA CCAATGCGCG TCGCACTGGC GATGTGAACG

          hinPI
          hhaI/cfoI                                                                         nlaIV
       rmaI                                                  mspI                          hgiJII
  hinPI  haeII                                               hpaII                         bsp1286
  hhaI/cfoI   bsrBI                                          naeI                          bmyI
 haeII maeI   aciI             mboII                 maeII   cfr10I          aluI          banII       nlaIV
4501 CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG
     GTCGCGGGAT CGCGGGCGAG GAAAGCGAAA GAAGGGAAGG AAAGAGCGGT GCAAGCGGCC GAAAGGGGCA GTTCGAGATT TAGCCCCCGA GGGAAATCCC

                 nlaIV                                              maeII   haeIII/palI
                 hgiCI  taqI                                       draIII  sau96I
                 banI mnlI                             hphI        bsaAI   asuI
4601 TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTTGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT
     AAGGCTAAAT CACGAAATGC CGTGGAGCTG GGGTTTTTTG AACTAAACCC ACTACCAAGT GCATCACCCG GTAGCGGGAC TATCTGCCAA AAAGCGGGAA

  maeII pleI         tru9I     pleI                        bslI
 drdI   hinfI  maeII mseI      hinfI        bsrI          bslI  avaI
4701 TGACGTTGGA GTCCACGTTC TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA ACCCTATCTC GGGCTATTCT TTTGATTTAT AAGGGATTTT
     ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACAA GGTTTGACCT TGTTGTGAGT TGGGATAGAG CCCGATAAGA AAACTAAATA TTCCCTAAAA
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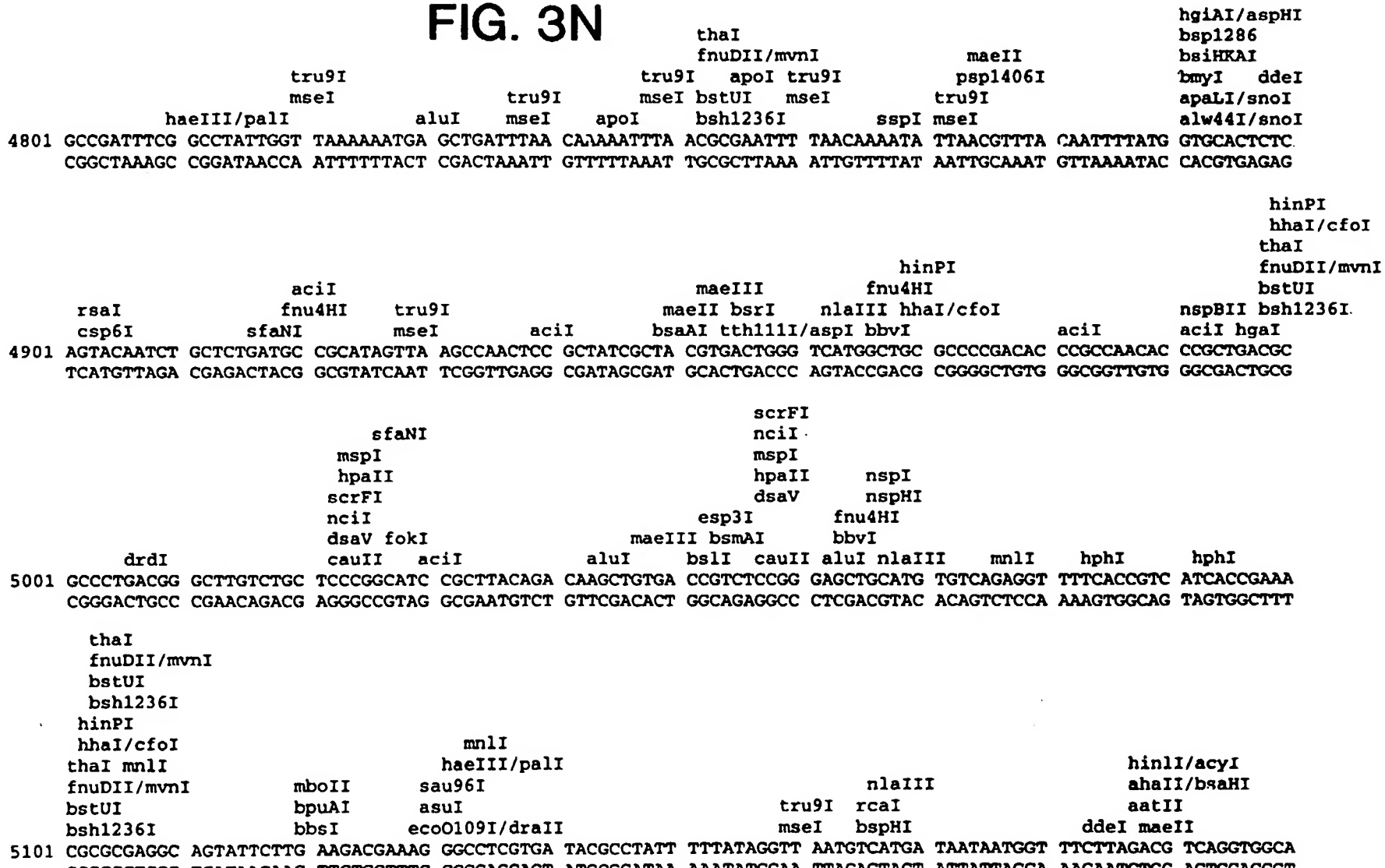
FIG. 3N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bmyI ddeI
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alw44I/snoI
thaI
fnuDII/mvnI
maeII
psp1406I
tru9I
mseI
tru9I
tru9I apoI tru9I
mseI bstUI mseI
tru9I
haeIII/palI
aluI
mseI
apoI
bsh1236I
sspI mseI
4801 GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTATG GTGCACTCTC
CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT GTTTTTAAAT TGCGCTTAAA ATTGTTTTAT AATTGCAAAT GTTAAAATAC CACGTGAGAG
hinPI
hhaI/cfoI
thaI
fnuDII/mvnI
bstUI
hinPI
aciI
maeIII
fnu4HI
rsaI
fnu4HI
tru9I
maeII bsrI
nlaIII hhaI/cfoI
nspBII bsh1236I
csp6I
sfaNI
mseI
aciI
bsaAI tth111I/aspI bbvI
aciI
aciI hgaI
4901 AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAACTCC GCTATCGCTA CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC
TCATGTTAGA CGAGACTACG GCGTATCAAT TCGGTTGAGG CGATAGCGAT GCACTGACCC AGTACCGACG CGGGGCTGTG GGCGGTTGTG GGCGACTGCG
scrFI
sfaNI
nciI
mspI
mspI
hpaII
hpaII
nspI
scrFI
dsaV
nspHI
nciI
esp3I
fnu4HI
dsaV fokI
maeIII bsmAI
bbvI
drdI
cauII aciI
aluI
bslI cauII aluI nlaIII
mnlI
hphI
hphI
5001 GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT TTTCACCGTC ATCACCGAAA
CGGGACTGCC CGAACAGACG AGGGCCGTAG GCGAATGTCT GTTCGACACT GGCAGAGGCC CTCGACGTAC ACAGTCTCCA AAAGTGGCAG TAGTGGCTTT
thaI
fnuDII/mvnI
bstUI
bsh1236I
hinPI
hhaI/cfoI
mnlI
thaI mnlI
haeIII/palI
hinlI/acyI
fnuDII/mvnI
mboII
sau96I
nlaIII
ahaII/bsaHI
bstUI
bpuAI
asuI
tru9I rcaI
aatII
bsh1236I
bbsI
eco0109I/draII
mseI bspHI
ddeI maeII
5101 CGCGCGAGGC AGTATTCTTG AAGACGAAAG GGCCTCGTGA TACGCCTATT TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA
GCGCGCTCCG TCATAAGAAC TTCTGCTTTC CCGGAGCACT ATGCGGATAA AAATATCCAA TTACAGTACT ATTATTACCA AAGAATCTGC AGTCCACCGT

FIG. 3O

```
                nlaIV
              aciI
             thaI
             fnuDII/mvnI
             bstUI
             bsh1236I
            hinPI                                                            bsrBI    bsmAI
            hhaI/cfoI                                                         aciI  nlaIII
                                                                                  rcaI
                                                                                  bspHI
5201 CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA
     GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT

                mboII                                                fnu4HI
  sspI          earI/ksp632I                                         aciI                         hphI
5301 ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
     TATTATAACT TTTTCCTTCT CATACTCATA AGTTGTAAAG GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT

                                          hgiAI/aspHI
                                          bsp1286
                              sau3AI      bsiHKAI                   sau3AI    nspBII      sau3AI
                              mboI/ndeII[dam-]                      mboI/ndeII[dam-]      mboI/ndeII[dam-]
                              dpnI[dam+]  bmyI                      dpnI[dam+]            dpnI[dam+]
                              dpnII[dam-]                          bstYI/xhoII            dpnII[dam-]
                          eco57I          apaLI/snoI            bsrI dpnII[dam-]          alwI[dam-]
      hphI            sfaNI  mboII[dam-]  alw44I/snoI  maeIII taqI  alwI[dam-]  aciI  bstYI/xhoII
5401 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC
     TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC GTGCTCACCC AATGTAGCTT GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG

                                                                                 scrFI
                                                     aciI                        nciI
                                                   thaI                          mspI
        maeII                                      fnuDII/mvnI                   hpaII
       psp1406I              hgiAI/aspHI           bstUI                         dsaV
      xmnI                   bsp1286  tru9I        bsh1236I                  hinlI/acyI
      asp700                 bsiHKAI  mseI        hinPI                      hgaI cauII              aciI
   mboII                     bmyI   ahaIII/draI   hhaI/cfoI                  ahaII/bsaHI   bcgI  mcrI  fnu4HI
5501 CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT
     GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA CGATACACCG CGCCATAATA GGGCACTACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA

                              rsaI
                              csp6I  bsrI                                                        fnu4HI
       ddeI                  scaI  hphI  maeIII     sfaNI     fokI   nlaIII                      bbvI        nlaIII
5601 ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC
     TGTGATAAGA GTCTTACTGA ACCAACTCAT GAGTGGTCAG TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG
```

FIG. 3P

```
                                                   sau96I
                                                   avaII
                                           sau3AI  asuI                                                   nlaIII
                haeIII/palI                mboI/ndeII[dam-]                                           sau3AI maeIII
                  eaeI                     dpnI[dam+]                                                 mboI/ndeII[dam-]
                  cfrI                     dpnII[dam-]                                                dpnI[dam+]
                 fnu4HI                   pvuI/bspCI                                                  dpnII[dam-]
                 aciI                     mcrI    mnlI        aluI     aciI          nlaIII alwI[dam-]
5701 ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC
     TACTCACTAT TGTGACGCCG GTTGAATGAA GACTGTTGCT AGCCTCCTGG CTTCCTCGAT TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG

                                                                                                   hinPI
                mspI                                                                               hhaI/cfoI
  sau3AI    nlaIV                                                                                 mstI
  mboI/ndeII[dam-] aluI                                                                           aviII/fspI    bsrI
  dpnI[dam+]    hpaII                                                          fnu4HI      maeII         tru9I
  dpnII[dam-] bsaWI                                          maeIII     sfaNI  bbvI      psp1406I        mseI
5801 TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC
     AACTAGCAAC CCTTGGCCTC GACTTACTTC GGTATGGTTT GCTGCTCGCA CTGTGGTGCT ACGGTCGTCG TTACCGTTGT TGCAACGCGT TTGATAATTG

                          mspI
                          hpaII                                                                    bglI
                         scrFI                                                                     sau96I
                  aluI   nciI         tru9I      fokI                           sau96I             haeIII/palI
                 rmaI    dsaV         mseI     bsrI        aciI                 avaII        hinPI  asuI     mspI
                 maeI    cauII       aseI/asnI/vspI    mnlI                     asuI         hhaI/cfoI       hpaII
5901 TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC
     ACCGCTTGAT GAATGAGATC GAAGGGCCGT TGTTAATTAT CTGACCTACC TCCGCCTATT TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG

                           mspI               thaI
                           hpaII              fnuDII/mvnI             haeIII/palI
                          cfr10I              bstUI                  sau96I
                        nlaIV hphI         bsmAI aciI          fnu4HI  nlaIV
                      gsuI/bpmI            bsaI  bsh1236I      bbvI bsrI asuI              mnlI
6001 TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT
     ACCAAATAAC GACTATTTAG ACCTCGGCCA CTCGCACCCA GAGCGCCATA GTAACGTCGT GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA

                                                   ddeI
                                               sau3AI       nlaIV
            pleI                               mboI/ndeII[dam-]
            hinfI                              dpnI[dam+]   hgiCI        tru9I
       eam1105I             fokI               dpnII[dam-]  banI mnlI    mseI        maeIII
6101 ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA
     TGTGCTGCCC CTCAGTCCGT TGATACCTAC TTGCTTTATC TGTCTAGCGA CTCTATCCAC GGAGTGACTA ATTCGTAACC ATTGACAGTC TGGTTCAAAT
```

FIG. 3Q

```
                                                                           rmaI        sau3AI
                                                                       sau3AI hphI    mboI/ndeII[dam-]
                                                                       mboI/ndeII[dam-]
                                                                       dpnI[dam+]     dpnI[dam+]
                                                                       dpnII[dam+]    dpnII[dam-]
                                         tru9I                   tru9I bstYI/xhoII    alwI[dam-]          nlaIII                maeII
                                         mseI            tru9I mseI   alwI[dam-]   bstYI/xhoII           rcaI               tru9I
                                       ahaIII/draI       mseI ahaIII/draI maeI   mboII[dam-]            bspHI              mseI
6201 CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT
     GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA

                                                                    sau3AI
                                                                    mboI/ndeII[dam-]
                                                                    dpnI[dam+]   sau3AI
                                                                    dpnII[dam-]  mboI/ndeII[dam-]  thaI
                                                                   bstYI/xhoII   dpnI[dam+]        fnuDII/mvnI
                                                         sau3AI    alwI[dam-]    dpnII[dam-]       bstUI
                                                         mboI/ndeII[dam-]        alwI[dam-]        bsh1236I
                                  hgaI                   dpnI[dam+] mboII[dam-]                   hinPI        fnu4HI
                              ddeI                       dpnII[dam-]           bstYI/xhoII        hhaI/cfoI    bbvI
6301 GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA
     CTCAAAAGCA AGGTGACTCG CAGTCTGGGG CATCTTTTCT AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT

                                                   sau3AI
                                                   mboI/ndeII[dam-]
                                                   dpnI[dam+]
                                                   dpnII[dam-]
                                                  alwI[dam-]
                               aciI             mspI                                     bsrI                  hinPI
                aciI        nspBII              hpaII      aluI                      maeIII      eco57I        hhaI/cfoI
6401 AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT
     TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT CGATGGTTGA GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA

                                                                                                                   fnu4HI
                                                                                                          alwNI    bbvI
               rmaI                    haeIII/palI                                                       bsrI  fnu4HI
               maeI       bslI        haeI                  scfI    aciI          mnlI             maeIII     bbvI      bsrI
6501 CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT
     GGAAGATCAC ATCGGCATCA ATCCGGTGGT GAAGTTCTTG AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA
```

FIG. 3R


scrFI
nciI
mspI
hpaII
dsaV
cauII
pleI
hinfI
mspI
hpaII
bsaWI
maeIII
aciI
nspBII
fnu4HI
bbvI
hinPI
mcrI
hhaI/cfoI
hgiAI/aspHI
bsp1286
bsiHKAI
bmyI
apaLI/snoI
alw44I/snoI
aluI
6601 GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT
CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA TCAATGGCCT ATTCCGCGTC GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA
mspI
hpaII
bslI
fnu4HI
hinPI
hhaI/cfoI
ddeI
scfI
haeII
aciI
bsaWI
aciI
6701 TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
ACCTCGCTTG CTGGATGTGG CTTGACTCTA TGGATGTCGC ACTCGTAACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC
scrFI
mvaI
ecoRII
dsaV
bstNI
bsaJI
scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
hinPI mnlI
hhaI/cfoI
aluI
apyI[dcm+]
taqI
mnlI drdI
hgaI
6801 CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT
GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT CCCCCTTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA
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haeIII/palI
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aciI
thaI bslI
fnuDII/mvnI
bstUI
bsh1236I
scrFI
mvaI bslI
ecoRII
dsaV
bstNI
apyI[dcm+]
haeIII/palI
haeI
nlaIII
nspI
nspHI
aflIII
nlaIV
sfaNI
aciI
nlaIV
haeI
6901 CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA
GCTAAAAACA CTACGAGCAG TCCCCCCGCC TCGGATACCT TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT
fnu4HI
bbvI
fnu4HI
bbvI pleI
hinPI hinfI
tfiI
hinfI
aciI
aluI
bsrBI
aciI
fnu4HI
mcrI
hhaI/cfoI
7001 TGTTCTTTCC TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA
ACAAGAAAGG ACGCAATAGG GGACTAAGAC ACCTATTGGC ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT

FIG. 3S

```
                                                                 thaI
                                                                 fnuDII/mvnI
                                                                 bstUI
                                                                 bsh1236I
                                                                hinPI
                                                                hhaI/cfoI
                                                               thaI
                                                               fnuDII/mvnI
                         sapI  hinPI                           bstUI   haeIII/palI        aluI
                         mboII hhaI/cfoI                       bsh1236I          tru9I  pvuII
                         earI/ksp632I               mnlI      bslI    eaeI  tfiI aseI/asnI/vspI
               mnlI  aciI    haeII               aciI         aciI    cfrI  hinfI mseI   nspBII               bsrI
   7101 GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATCCAGCT GGCACGACAG GTTTCCCGAC
        CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT GGCGGAGAGG GGCGCGCAAC CGGCTAAGTA ATTAGGTCGA CCGTGCTGTC CAAAGGGCTG

                                                                    scrFI
                                                                    mvaI
                                                                    ecoRII
                                                                    dsaV
                                      tru9I                    nlaIV bstNI
                        hinPI         mseI       maeIII        hgiCI apyI[dcm+]                mspI
           aciI         hhaI/cfoI    aseI/asnI/vspI  mnlI      banI bsaJI                      hpaII
   7201 TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG
        ACCTTTCGCC CGTCACTCGC GTTGCGTTAA TTACACTCAA TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC

                                                                 tru9I
                                                                 mseI
                                                                aseI/asnI/vspI
              aciI                                             xmnI
            bsrBI                        aluI      nlaIII      asp700
   7301 GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGAATTAA
        CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACTGGTACTA ATGCTTAATT

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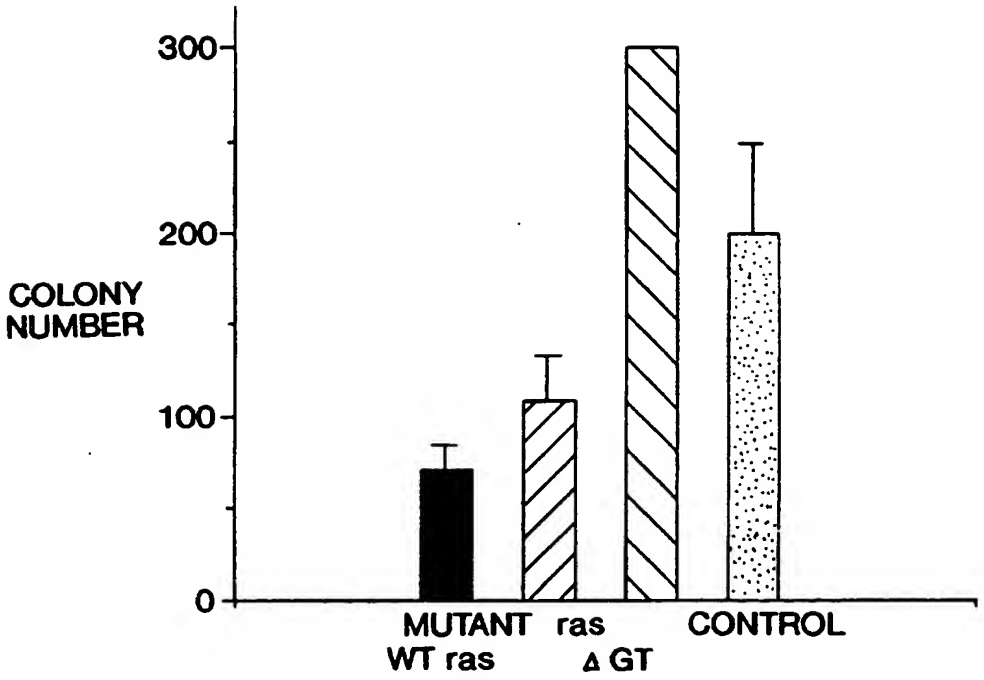
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100
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MUTANT ras
WT ras
Δ GT
CONTROL

FIG. 4

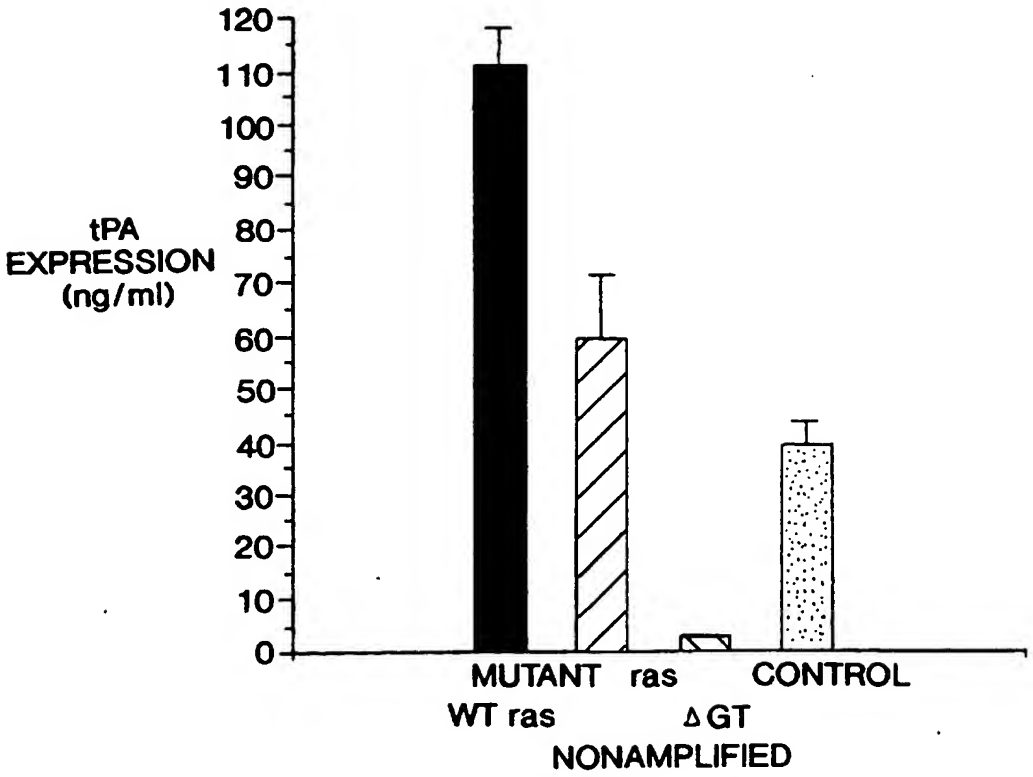
120
110
100
90
80
70
60
50
40
30
20
10
0
tPA EXPRESSION (ng/ml)
MUTANT ras
WT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5A

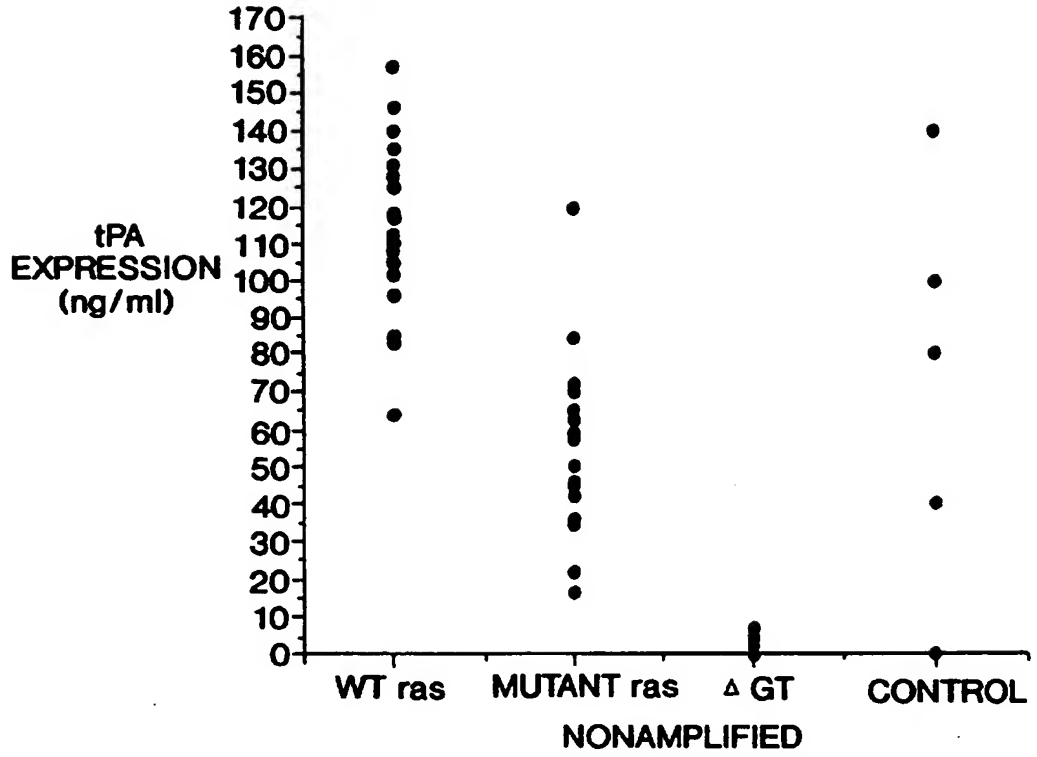
tPA
EXPRESSION
(ng/ml)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
WT ras
MUTANT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5B

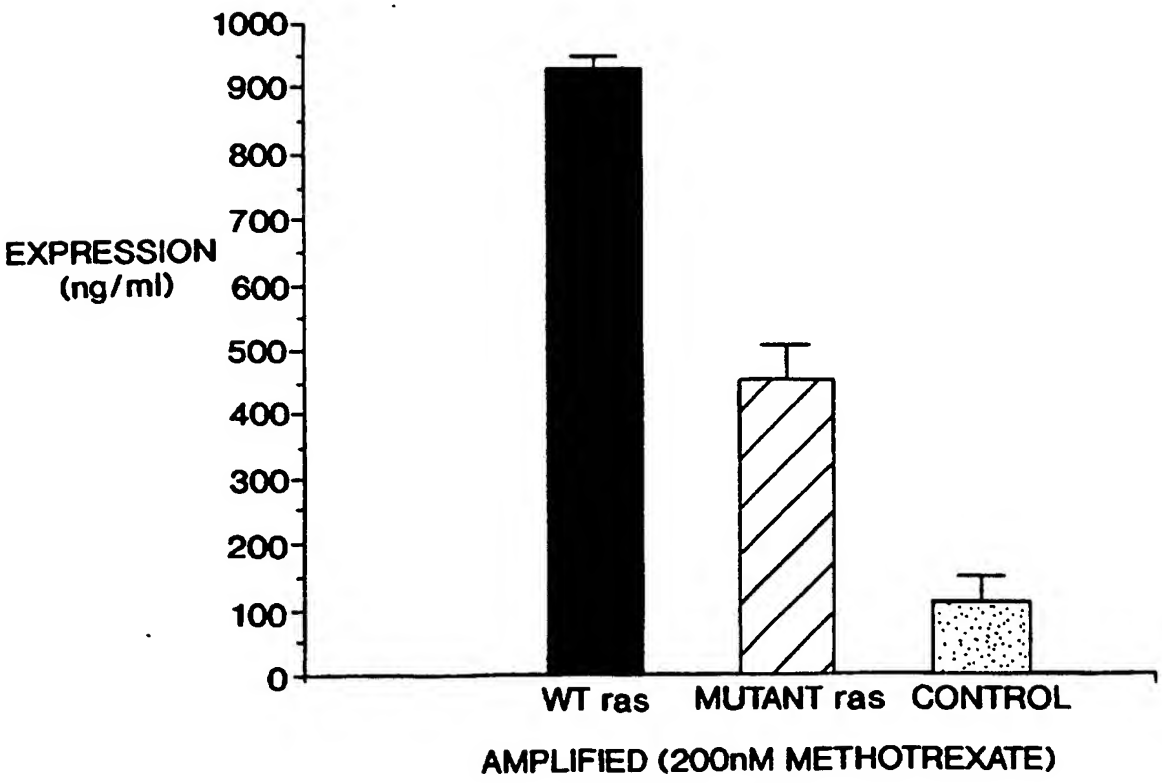
EXPRESSION
(ng/ml)
1000
900
800
700
600
500
400
300
200
100
0
WT ras
MUTANT ras
CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 5C

FIG. 6A

```
      aluI
     sstI
     sacI
     hgiJII
     hgiAI/aspHI
     ecl136II
     bsp1286
     bsiHKAI
     bmyI                                rmaI    tru9I                                                                   thaI
     banII                               maeI    mseI                                                                    fnuDII/mvnI
    taqI                                speI    aseI/asnI/vspI                               bslI                        bstUI
                                                                                                                         bsh1236I
                                                                                                                        aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                  scrFI
                  mvaI
                  ecoRII
                  dsaV
               aciI
             bglI bstNI                                        maeII
            sau96I                                            hinlI/acyI
            haeIII/palI    aciI                               ahaII/bsaHI
            asuI  apyI[dcm+]                     aciI         aatII        maeII           maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

     maeII                                                                                                  maeII
    hinlI/acyI                                                                                             hinlI/acyI
    ahaII/bsaHI                                        rsaI                          rsaI                  ahaII/bsaHI
    aatII                                    bglI      csp6I            ndeI         csp6I                 aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII                                                                                         nlaIII
        aciI                                                                                            styI
      bglI  dsaV                                                                                              ncoI
     sau96I bstNI          rsaI                                                  maeII                        dsaI hphI  aciI
     haeIII/palI           csp6I                                          rsaI   snaBI                        bsaJI   sfaNI
     asuI   apyI[dcm+]   bsrI  nlaIII                                     csp6I  bsaAI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 6B

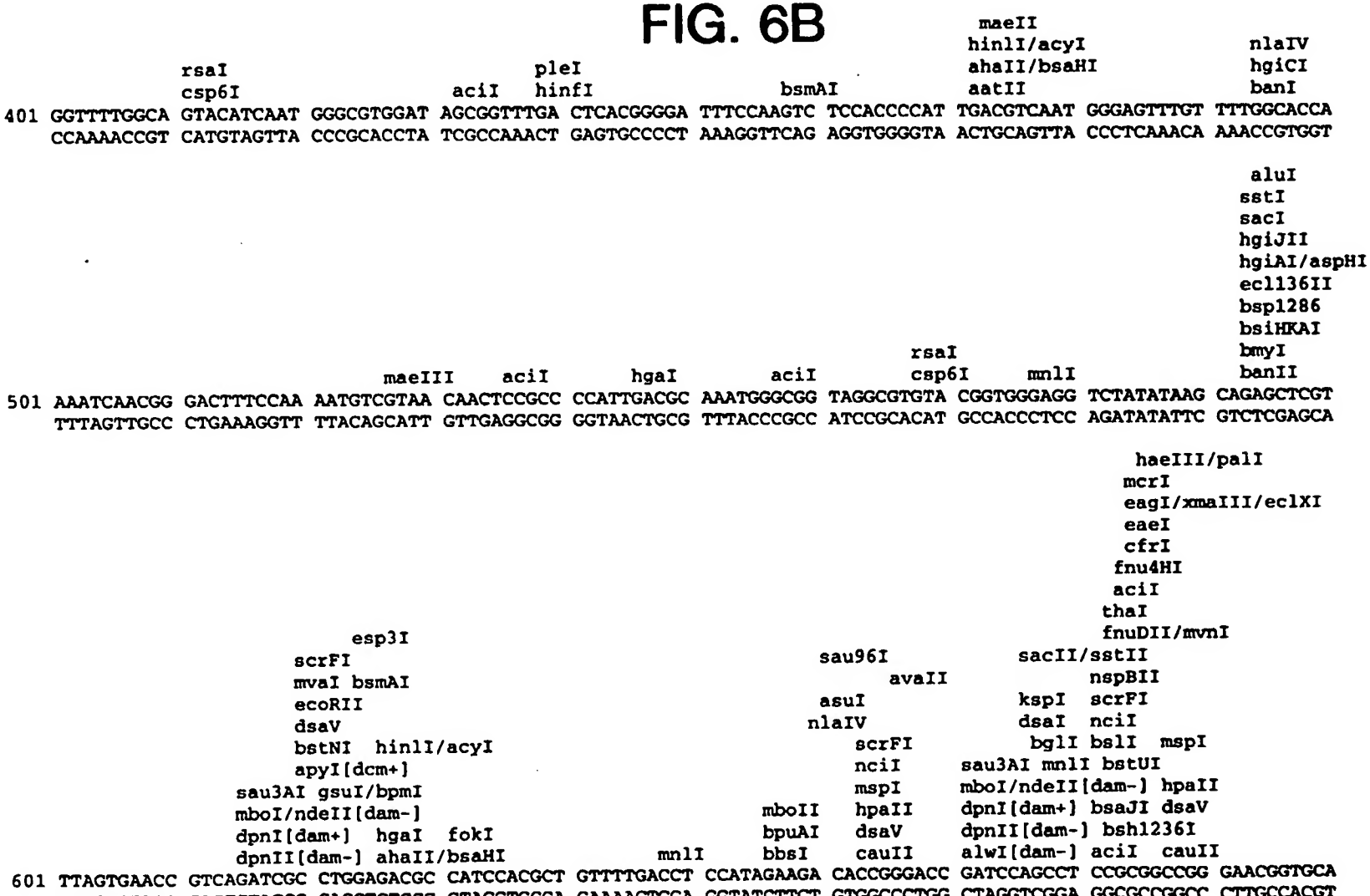
maeII
hinII/acyI
nlaIV
rsaI
pleI
ahaII/bsaHI
hgiCI
csp6I
aciI
hinfI
bsmAI
aatII
banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT
aluI
sstI
sacI
hgiJII
hgiAI/aspHI
ecl136II
bsp1286
bsiHKAI
rsaI
bmyI
maeIII
aciI
hgaI
aciI
csp6I
mnlI
banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA
haeIII/palI
mcrI
eagI/xmaIII/eclXI
eaeI
cfrI
fnu4HI
aciI
thaI
fnuDII/mvnI
esp3I
scrFI
sau96I
sacII/sstII
mvaI bsmAI
avaII
nspBII
ecoRII
asuI
kspI scrFI
dsaV
nlaIV
dsaI nciI
bstNI hinII/acyI
scrFI
bglI bslI mspI
apyI[dcm+]
nciI
sau3AI mnlI bstUI
sau3AI gsuI/bpmI
mspI
mboI/ndeII[dam-] hpaII
mboI/ndeII[dam-]
mboII
hpaII
dpnI[dam+] bsaJI dsaV
dpnI[dam+] hgaI fokI
bpuAI
dsaV
dpnII[dam-] bsh1236I
dpnII[dam-] ahaII/bsaHI
mnlI
bbsI
cauII
alwI[dam-] aciI cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT

FIG. 6C

```
              tfiI
           aciI
          thaI hinfI                                                fnu4HI
          fnuDII/mvnI                  aciI                         bbvI
          bstUI                     rsaI                          nspBII        taqI
          bsh1236I                  csp6I  scfI       mnlI        aciI     nlaIII
701  TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                  thaI
                                                                  fnuDII/mvnI
                                                                  bstUI
                                                                  bsh1236I
                                                                 mluI
                                                        bsrBI    aflIII   rsaI
                   pflMI                 bsmAI          aciI   xmnI       csp6I
     sfaNI         bslI                  bsaI        mnlI ddeI asp700    scaI
801  GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
     CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                 scrFI
                                                 mvaI
                                                 ecoRII
                                                 dsaV
         eco57I
        mboII             tfiI                   bstNI                ::   tfiI     tru9I      tru9I
       earI/ksp632I       hinfI                  apyI[dcm+]      mboII taqI     mseI       mseI
      mnlI              alwNI   hphI           sexAI          ddeI  hinfI   ahaIII/draI aseI/asnI/vspI
901  AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
     TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                     aluI
                                     sstI
                                     sacI
                                     hgiJII
                                     hgiAI/aspHI
                                     ecl136II
                                     bsp1286
                                     bsiHKAI
                                     bmyI                          tru9I           mspI
                                     banII                    sfaNI mseI           hpaII
          ddeI              bslI   mnlI           bstXI    fokI    aflII/bfrI     bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 6D

```
                                                                               haeIII/palI
                                                                              haeI
                                                       scrFI          scrFI                       nlaIII
                                                       mvaI                 mvaI                           sau3AI
                                                       ecoRII         ecoRII                   mboI/ndeII[dam-]
                                                       dsaV   tfiI    dsaV    pleI             dpnI[dam+]
                                                       bstNI     nlaIII     bstNI     ddeI                 dpnII[dam-]
             accI  nlaIII             mnlI             apyI[dcm+]   hinfI   apyI[dcm+]    hinfI   maeIII  alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                          mnlI
                                                                                     hinlI/acyI         scrFI
                                                                                     ahaII/bsaHI        mvaI
                                                                                  scrFI          ecoRII
                                                                                  mvaI    ecoNI         dsaV
                                                                                  ecoRII      sau96I
                                                                                  dsaV        avaII
                         maeII                                                    bstNI   bslI      asuI    mnlI
                       aflIII                                                     apyI[dcm+]     mnlI  bstNI
         apoI        maeIII                                      mnlI           bsaJI hgaI   ddeI      apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                                  ppu10I
                                                             sfaNI                                nsiI/avaIII
         sfaNI                accI     mboII            mboII                            mnlI     aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                         fnu4HI
        nlaIII                                           aciI
       styI                                             thaI
       ncoI                                             fnuDII/mvnI tru9I
       dsaI                           styI              bstUI       mseI                                 maeIII
       bsaJI                          bsaJI             bsh1236I   aseI/asnI/vspI                       hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 6E

```
                                                                                          haeIII/palI
                                                                                         eaeI
                                                 scrFI                              cfrI
                                                 mvaI                                    mspI
                                                 ecoRII                            hpaII
                                                 dsaV                             scrFI
                                                 bstNI                                  nciI
                                                 apyI[dcm+]      mnlI            ecoRI  dsaV
                                               gsuI/bpmI         bsaJI      taqI apoI   cauII
        scfI        fokI                   maeIII    hincII/hindII         claI/bsp106 bsaJI      aluI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATAGCTAACT TAAGGGGCCG GTATCGACAG

                                                   gsuI/bpmI
                                                  scrFI
                                                  mvaI
                                                  ecoRII                                              mnlI
                                                  dsaV                                                ecoNI
            mnlI                         nspBII   bstNI                         ddeI                    sau96I
          haeIII/palI               fnu4HI     hgiAI/aspHI                     mnlI                     nlaIV
         sau96I                  fnu4HI aciI   bsp1286                         eco81I                   avaII
         asuI                    bbvI  fnu4HI  bsiHKAI     aluI                bsu36I/mstII/sauI        asuI   hphI
      nlaIII                 bspMI    bbvI     bmyI apyI[dcm+]               bslI                     bsrI   bslI
1601 TGGCATGGGC CTCTCCACCG TGCCTGACCT GCTGCTGCCG CTGGTGCTCC TGGAGCTGTT GGTGGGAATA TACCCCTCAG GGGTTATTGG ACTGGTCCCT
     ACCGTACCCG GAGAGGTGGC ACGGACTGGA CGACGACGGC GACCACGAGG ACCTCGACAA CCACCCTTAT ATGGGGAGTC CCCAATAACC TGACCAGGGA

      rmaI
      maeI
     styI
     bsaJI
     blnI           mboII                  styI                                     rsaI
     avrII          earI/ksp632I           bsaJI            mnlI       taqI         csp6I               nlaIV
1701 CACCTAGGGG ACAGGGAGAA GAGAGATAGT GTGTGTCCCC AAGGAAAATA TATCCACCCT CAAAATAATT CGATTTGCTG TACCAAGTGC CACAAAGGAA
     GTGGATCCCC TGTCCCTCTT CTCTCTATCA CACACAGGGG TTCCTTTTAT ATAGGTGGGA GTTTTATTAA GCTAAACGAC ATGGTTCACG GTGTTTCCTT
```

FIG. 6F

```
                                 scrFI
                                 nciI
                                 mspI
                                 hpaII
                                 dsaV
                                 cauII
                                xmaI/pspAI
                                smaI
                                scrFI
                                nciI
                                dsaV
                                cauII
                                bslI
                              sau96I
                              haeIII/palI
                              asuI
                           scrFI
                           mvaI bsaJI
                           ecoRII
                           dsaV                                            nlaIV
           rsaI .          bstNI bsaJI          scfI                          fnu4HI                                   alwNI
            csp6I          bslI avaI            pstI                          aciI         aciI                 ddeI
          bsp1407I         apyI[dcm+]           bsgI                       bsrBI      hphI   eco57I          mnlI
1801 CCTACTTGTA CAATGACTGT CCAGGCCCGG GGCAGGATAC GGACTGCAGG GAGTGTGAGA GCGGCTCCTT CACCGCTTCA GAAAACCACC TCAGACACTG
     GGATGAACAT GTTACTGACA GGTCCGGGCC CCGTCCTATG CCTGACGTCC CTCACACTCT CGCCGAGGAA GTGGCGAAGT CTTTTGGTGG AGTCTGTGAC

                                                                                 scrFI
                                                   mboII                         nciI
                                                  earI/ksp632I                   mspI
         fnu4HI                                  sau3AI                          hpaII
        aluI                                     mboI/ndeII[dam-]                dsaV                 scfI                mspI
       pvuII                                     dpnI[dam+]                      cauII                pstI                hpaII
       nspBII                                    dpnII[dam-]                  sau96I                  bsgI             rsaI
     ddeI                                       bstYI/xhoII                   avaII                  fnu4HI            csp6I
     mnlI bbvI                                  bglII                         asuI     draIII        bbvI   mboII   bsrI cfr10I
1901 CCTCAGCTGC TCCAAATGCC GAAAGGAAAT GGGTCAGGTG GAGATCTCTT CTTGCACAGT GGACCGGGAC ACCGTGTGTG GCTGCAGGAA GAACCAGTAC
     GGAGTCGACG AGGTTTACGG CTTTCCTTTA CCCAGTCCAC CTCTAGAGAA GAACGTGTCA CCTGGCCCTG TGGCACACAC CGACGTCCTT CTTGGTCATG
```

FIG. 6G

FIG. 6H

```
                                                                  eam1105I
                                                                 sau96I
                                                        scrFI
                                  bsp1286               mvaI     avaII
                                 nlaIV                  ecoRII
                                 hgiCI                  dsaV
                             dsaI bmyI                  bstNI    asuI       mboII mboII
                             bsaJI                      bsaJI mnlI         bpuAI earI/ksp632I      styI
   maeIII   mnlI   nlaIII    banI    alwNI              apyI[dcm+]         bbsI mnlI               bsaJI
2401 CTTGTGACAC ACCTCCCCCA TGCCCACGGT GCCCAGCACC TGAACTCCTG GGAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ATACCCTTAT
     GAACACTGTG TGGAGGGGGT ACGGGTGCCA CGGGTCGTGG ACTTGAGGAC CCTCCTGGCA GTCAGAAGGA GAAGGGGGGT TTTGGGTTCC TATGGGAATA

        sau96I
        nlaIV
        avaII
        asuI            maeII
      mspI             pmlI
      hpaII            eco72I                                      sau96I
     scrFI      mnlI   bsaAI                            drdI       avaII             maeII
     nciI     ddeI maeIII                           mboII      mnlI                 bsaAI
     dsaV    eco81I    bbrPI                        bpuAI    bsaJI   bsrI          rsaI
     cauII   bsu36I/mstII/sauI        maeII         bbsI    avaI   asuI            csp6I          mnlI
2501 GATTTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC GTGAGCCACG AAGACCCCGA GGTCCAGTTC AAGTGGTACG TGGACGGCGT GGAGGTGCAT
     CTAAAGGGCC TGGGGACTCC AGTGCACGCA CCACCACCTG CACTCGGTGC TTCTGGGGCT CCAGGTCAAG TTCACCATGC ACCTGCCGCA CCTCCACGTA

               aciI
              thaI
              fnuDII/mvnI
              bstUI
              bsh1236I
             sacII/sstII
             nspBII                                                               scrFI
             kspI                                                                 mvaI bsrI
             dsaI                                                                 ecoRII
             bsaJI                                                                dsaV
             aciI                                        mnlI       ecoNI        bstNI                  rsaI
            fnu4HI  mnlI            maeII             hgaI    hphI  bslI        apyI[dcm+]              csp6I
2601 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TTCAACAGCA CGTTCCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAC GGCAAGGAGT
     TTACGGTTCT GTTTCGGCGC CCTCCTCGTC AAGTTGTCGT GCAAGGCACA CCAGTCGCAG GAGTGGCAGG ACGTGGTCCT GACCGACTTG CCGTTCCTCA
```

FIG. 6I

```
                                                                                                    rsaI
                                                                                                    csp6I
            bsmAI
           bsaI            mnlI           taqI                                  avaI            bsp1407I    bslI
2701 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAA CCAAAGGACA GCCCCGAGAA CCACAGGTGT ACACCCTGCC
     TGTTCACGTT CCAGAGGTTG TTTCGGGAGG GTCGGGGGTA GCTCTTTTGG TAGAGGTTTT GGTTTCCTGT CGGGGCTCTT GGTGTCCACA TGTGGGACGG

           scrFI
           nciI
           mspI
           hpaII
           dsaV
           cauII
          xmaI/pspAI
          smaI
          scrFI                  scrFI         scrFI
          nciI                   mvaI                mvaI
          dsaV                   ecoRII        ecoRII
          cauII                  dsaV          dsaV                                          aciI
          bsaJI                  bstNI               bstNI                           dsaI          nspBII
       fokI                      apyI[dcm+]          apyI[dcm+]                      bslI         fnu4HI fnu4HI
     bslI avaI  mnlI            sexAI           bspMI                                bsaJI        bbvI   bbvI
2801 CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG
     GGGTAGGGCC CTCCTCTACT GGTTCTTGGT CCAGTCGGAC TGGACGGACC AGTTTCCGAA GATGGGGTCG CTGTAGCGGC ACCTCACCCT CTCGTCGCCC

                                                                                    dsaI
        mspI                                  pleI                 mnlI          hphI                        fnu4HI
        hpaII                    mnlI   nlaIII hinfI     nlaIV   mboII   scfI    aluI  bsaJI        bspMI   bbvI
2901 CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC
     GTCGGCCTCT TGTTGATGTT GTGGTGCGGA GGGTACGACC TGAGGCTGCC GAGGAAGAAG GAGATGTCGT TCGAGTGGCA CCTGTTCTCG TCCACCGTCG

                                                                                                      scrFI
                                                                                                      nciI
                                                                                                      mspI
                                                                                                      hpaII
                                                                                                      dsaV
                                                                                     scrFI            cauII
                                                                                     nciI             bsaJI
                                                                                     mspI            haeIII/palI
                                  nlaIII                                             hpaII          mcrI
                               ppu10I                                sapI            dsaV           eagI/xmaIII/eclXI
         xmnI mboII            nsiI/avaIII                           mboII   mnlI   bsmAI           eaeI
         asp700     nlaIII     sfaNI   mnlI          aciI            earI/ksp632I bslI  cauII       cfrI
3001 AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC TTCACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGAG TGCGACGGCC
     TCCCCTTGTA GAAGAGTACG AGGCACTACG TACTCCGAGA CGTGTTGGCG AAGTGCGTCT TCTCGGAGAG GGACAGAGGC CCATTTACTC ACGCTGCCGG
```

FIG. 6J

```
                rmaI
              mnlI
            sau3AI
            mboI/ndeII[dam-]                    styI
            dpnI[dam+]                        aciI
            dpnII[dam-]  taqI                fnu4HI   sau96I
            alwI[dam-]  salI   scfI         sfiI ncoI haeIII/palI
           nlaIV  maeI  hincII/hindII       haeIII/palI
           bstYI/xhoII  accI   pstI        eaeI  dsaI asuI              aluI
           bamHI xbaI pleI     bsgI   aluI cfrI  bsaJI                fnu4HI
           alwI[dam-] hinfI bspMI    hindIII bglI nlaIII               bbvI          maeIII             sfaNI   apoI
3101 GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT
     CCCCTAGGAG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA TTTCGTTATC GTAGTGTTTA

                                                                                                    sau3AI
                                                                                                    mboI/ndeII[dam-]
                                                                                                    dpnI[dam+]
                                                                                                    dpnII[dam-]
                                                                                                   pvuI/bspCI
                                                                                                   mcrI
                                                                                                  taqI[dam-]   tru9I
                                                                                                 claI/bsp106[dam-]
                                                                                                sau3AI          mseI
                                                                                                mboI/ndeII[dam-]
                                                                                                dpnI[dam+] xmnI        hinPI
                                                                                                dpnII[dam-]  aseI/asnI/vspI
                                    rmaI
                                bsmI  maeI                                          nlaIII  alwI[dam-]  asp700      hhaI/cfoI
3201 TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC
     AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA GCTAGCCCTT AATTAAGCCG

                                                rsaI
                                                csp6I
           haeI                                nlaIV
         styI                                  kpnI
         ncoI                                  hgiCI
        dsaI haeIII/palI                       banI                      aluI
    fnu4HI nlaIII                              asp718     mnlI          pvuII
    bbvI  bsaJI             mnlI      mnlI     acc65I   ddeI aciI       nspBII
3301 GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG
     CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT ACACACAGTC AATCCCACAC
```

FIG. 6K

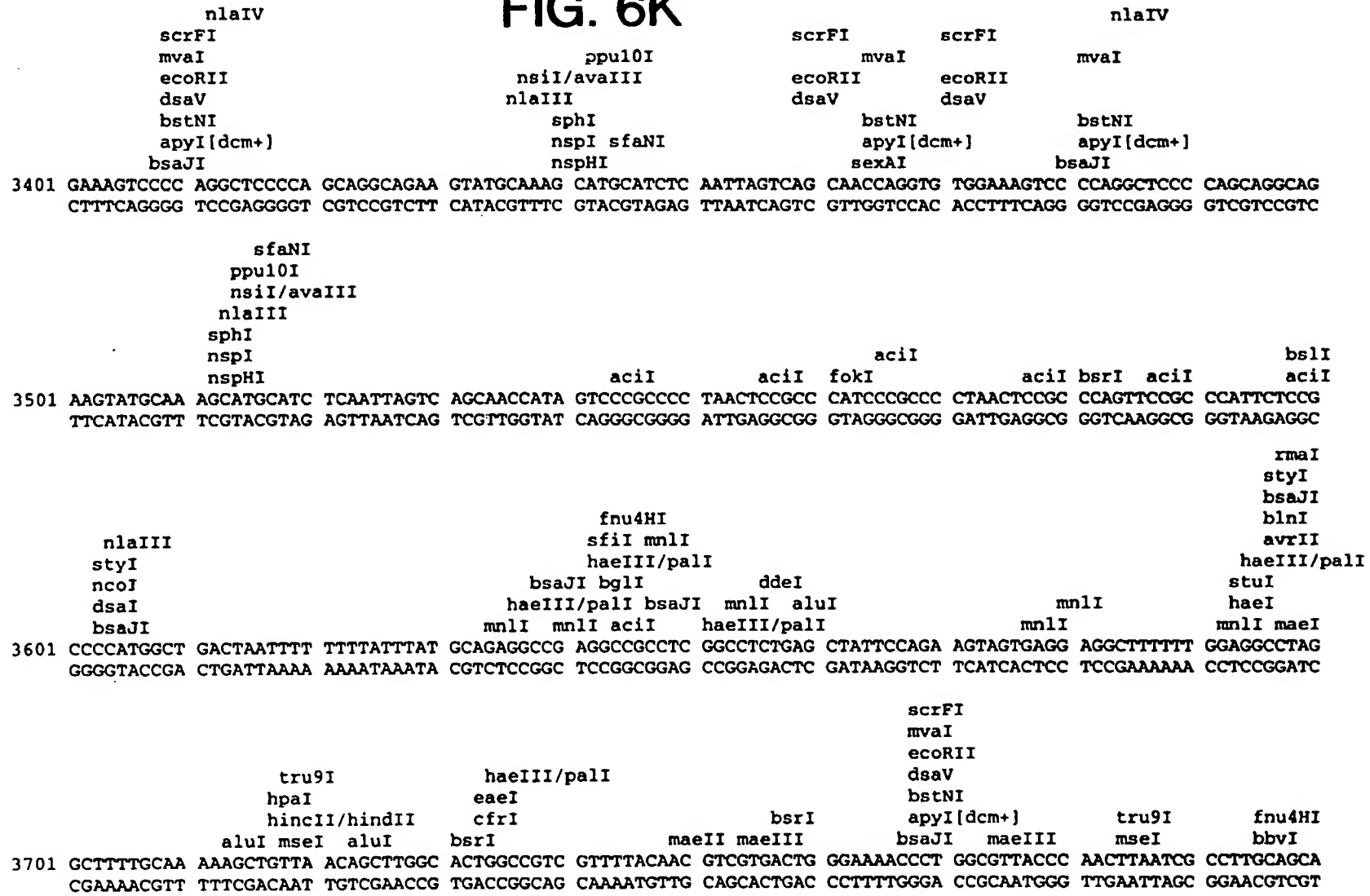

FIG. 6L

```
                                                                                                   hinPI
                                                                                                   hhaI/cfoI
                                                                                                  nlaIV
                                                      sau3AI                                      narI
                                           sau96I     mboI/ndeII[dam-]                            kasI
                                           haeIII/palI                                            hinlI/acyI
                                           asuI       dpnI[dam+]                                  hgiCI
                     aluI                mnlI         dpnII[dam-]                                 haeII      aciI
                    pvuII             mboII   aciI   pvuI/bspCI                                   banI    sfaNI
     fokI           nspBII            earI/ksp632I   mcrI                         bglI            ahaII/bsaHI
3801 CATCCCCCCT TCGCCAGCTG GCGTAATAGC GAAGAGGCCC GCACCGATCG CCCTTCCCAA CAGTTGCGTA GCCTGAATGG CGAATGGCGC CTGATGCGGT
     GTAGGGGGGA AGCGGTCGAC CGCATTATCG CTTCTCCGGG CGTGGCTAGC GGGAAGGGTT GTCAACGCAT CGGACTTACC GCTTACCGCG GACTACGCCA

                                                                                                     aciI
                                                                                                  fnu4HI
                                                                        hinPI                     aciI
                                                                       thaI                      thaI
                                                                       fnuDII/mvnI               fnuDII/mvnI
                                                                       bstUI scfI     hinPI      bstUI
                                                                       bsh1236I       hhaI/cfoI hinPI
                                                                   rsaI hhaI/cfoI  fnu4HI  tru9I bsh1236I
                   sfaNI    aciI          aciI   maeII                 csp6I   bslI    aciI    mseI hhaI/cfoI
3901 ATTTTCTCCT TACGCATCTG TGCGGTATTT CACACCGCAT ACGTCAAAGC AACCATAGTA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG
     TAAAAGAGGA ATGCGTAGAC ACGCCATAAA GTGTGGCGTA TGCAGTTTCG TTGGTATCAT GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC

            fnu4HI
          hinPI
          hhaI/cfoI                     hinPI
         thaI                           hhaI/cfoI
         fnuDII/mvnI                 rmaI                                                         mspI
         bstUI                 hinPI  haeII                                                      hpaII
         bsh1236I     aciI     hhaI/cfoI     bsrBI                                              naeI
     maeIII bbvI  maeIII      haeII maeI     aciI            mboII                       maeII  cfr10I
4001 GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC TTTCCCCGTC
     CAATGCGCGT CGCACTGGCG ATGTGAACGG TCGCGGGATC GCGGGCGAGG AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCG AAAGGGGCAG

                     nlaIV
                    hgiJII
                    bsp1286                                 nlaIV                                  maeII    haeIII/palI
                    bmyI                                    hgiCI  taqI                           draIII   sau96I
      aluI          banII         nlaIV                     banI mnlI                   hphI      bsaAI    asuI
4101 AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAAACT TGATTTGGGT GATGGTTCAC GTAGTGGGCC
     TTCGAGATTT AGCCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC GTGGAGCTGG GGTTTTTTGA ACTAAACCCA CTACCAAGTG CATCACCCGG
```

FIG. 6M

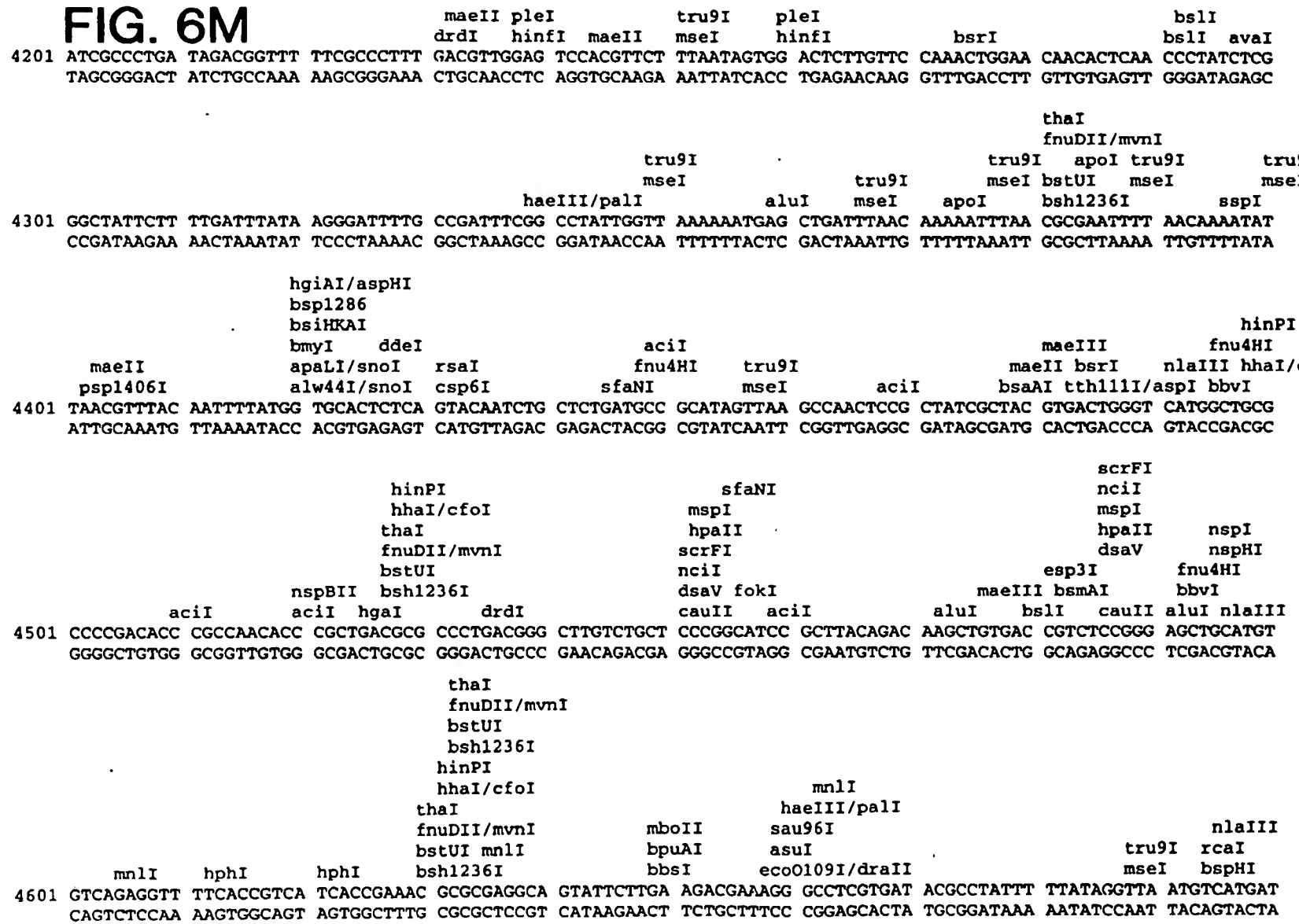

FIG. 6N

```
                                                          nlaIV
                                                        aciI
                                                       thaI
                                                       fnuDII/mvnI
                     hinlI/acyI                        bstUI                                                    rcaI
                     ahaII/bsaHI                       bsh1236I                                                 bspHI
                     aatII                            hinPI                                                 bsrBI
                 ddeI maeII                           hhaI/cfoI                                             aciI nlaIII
4701 AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC
     TTATTACCAA AGAATCTGCA GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA AAGATTTATG TAAGTTTATA CATAGGCGAG

                                                     mboII                                                  fnu4HI
       bsmAI                           sspI          earI/ksp632I                                           aciI
4801 ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT
     TACTCTGTTA TTGGGACTAT TTACGAAGTT ATTATAACTT TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA ATAAGGGAAA AAACGCCGTA

                                                                             hgiAI/aspHI
                                                                             bsp1286
                                                                  sau3AI     bsiHKAI                    sau3AI
                                                                  mboI/ndeII[dam-]                      mboI/ndeII[dam-]
                                                                  dpnI[dam+] bmyI                       dpnI[dam+]
                                                                  dpnII[dam-]                          bstYI/xhoII
                                                             eco57I          apaLI/snoI             bsrI dpnII[dam-]
                         hphI             hphI              sfaNI  mboII[dam-]   alw44I/snoI  maeIII taqI    alwI[dam-]
4901 TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA
     AAACGGAAGG ACAAAAACGA GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG TGCTCACCCA ATGTAGCTTG ACCTAGAGTT

                                                                                            aciI
              sau3AI                                                                       thaI
              mboI/ndeII[dam-]              maeII                                          fnuDII/mvnI
              dpnI[dam+]                   psp1406I             hgiAI/aspHI                bstUI
              dpnII[dam-]                 xmnI                  bsp1286 tru9I              bsh1236I                 hinlI/acyI
       aciI   alwI[dam-]                  asp700                bsiHKAI mseI              hinPI                     hgaI
     nspBII  bstYI/xhoII               mboII                    bmyI   ahaIII/draI        hhaI/cfoI                 ahaII/bsaHI
5001 CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC
     GTCGCCATTC TAGGAACTCT CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC GCCATAATAG GGCACTACTG

      scrFI
      nciI
      mspI
      hpaII                                                      rsaI
      dsaV                    aciI                               csp6I   bsrI
      cauII      bcgI  mcrI  fnu4HI         ddeI                scaI  hphI  maeIII      sfaNI     fokI   nlaIII
5101 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG
     CGGCCCGTTC TCGTTGAGCC AGCGGCGTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT AGAATGCCTA CCGTACTGTC
```

FIG. 6O

```
                                                                                                   sau96I
                                                                                                   avaII
                                                                                           sau3AI  asuI
                                                                     haeIII/palI           mboI/ndeII[dam-]
                                                                    eaeI                   dpnI[dam+]
                                                                    cfrI                   dpnII[dam-]
                       fnu4HI                                      fnu4HI                 pvuI/bspCI
                       bbvI        nlaIII                          aciI                   mcrI    mnlI         aluI    aciI
5201 TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT
     ATTCTCTTAA TACGTCACGA CGGTATTGGT ACTCACTATT GTGACGCCGG TTGAATGAAG ACTGTTGCTA GCCTCCTGGC TTCCTCGATT GGCGAAAAAA

                         maeIII
                      nlaIII
                   sau3AI                            mspI
                   mboI/ndeII[dam-]    sau3AI  nlaIV
                   dpnI[dam+]          mboI/ndeII[dam-] aluI
                   dpnII[dam-]         dpnI[dam+]    hpaII                                                fnu4HI
           nlaIII alwI[dam-]           dpnII[dam-] bsaWI                                maeIII     sfaNI  bbvI
5301 GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCAGCAGCA
     CGTGTTGTAC CCCCTAGTAC ATTGAGCGGA ACTAGCAACC CTTGGCCTCG ACTTACTTCG GTATGGTTTG CTGCTCGCAC TGTGGTGCTA CGGTCGTCGT

                                                                          mspI
                    hinPI                                                 hpaII
                   mstI                                                  scrFI
                   aviII/fspI     bsrI                          aluI  nciI        tru9I      fokI                sau96I
              maeII hhaI/cfoI   tru9I                          rmaI   dsaV        mseI    bsrI        aciI       avaII
             psp1406I           mseI                           maeI   cauII      aseI/asnI/vspI  mnlI             asuI
5401 ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC
     TACCGTTGTT GCAACGCGTT TGATAATTGA CCGCTTGATG AATGAGATCG AAGGGCCGTT GTTAATTATC TGACCTACCT CCGCCTATTT CAACGTCCTG

                    bglI                                                   mspI              thaI
                   sau96I                                                  hpaII             fnuDII/mvnI           haeIII/palI
                   haeIII/palI                                            cfr10I             bstUI                sau96I
           hinPI  asuI      mspI                                        nlaIV hphI       bsmAI aciI       fnu4HI  nlaIV
           hhaI/cfoI        hpaII                                     gsuI/bpmI          bsaI  bsh1236I   bbvI bsrI asuI
5501 CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA
     GTGAAGACGC GAGCCGGGAA GGCCGACCGA CCAAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG AGCGCCATAG TAACGTCGTG ACCCCGGTCT

                                                                                                  ddeI
                                                                                             sau3AI       nlaIV
                                                   pleI                                      mboI/ndeII[dam-]  mnlI
                                                   hinfI                                     dpnI[dam+]   hgiCI          tru9I
              mnlI                           eam1105I              fokI                      dpnII[dam-]  banI           mseI
5601 TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT
     ACCATTCGGG AGGGCATAGC ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT TGCTTTATCT GTCTAGCGAC TCTATCCACG GAGTGACTAA
```

FIG. 6P

```
                                                                                          rmaI      sau3AI
                                                                                      sau3AI hphI  mboI/ndeII[dam-]
                                                                                      mboI/ndeII[dam-]
                                                                                      dpnI[dam+]   dpnI[dam+]
                                                                                      dpnII[dam-]  dpnII[dam-]
                                                               tru9I          ahaIII/draI  maeI      alwI[dam-]
                                                               mseI       tru9I tru9I bstYI/xhoII  bstYI/xhoII
             maeIII                                            ahaIII/draI   mseI  mseI  alwI[dam-] mboII[dam-]
5701 AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG
     TTCGTAACCA TTGACAGTCT GGTTCAAATG AGTATATATG AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC

                                                                                    sau3AI
                                                                                    mboI/ndeII[dam-]
                                                                                    dpnI[dam+]  sau3AI
                                                                                    dpnII[dam-] mboI/ndeII[dam-]
                                                                                   bstYI/xhoII  dpnI[dam+]
                                                                            sau3AI  alwI[dam-]  dpnII[dam-]
                                                                            mboI/ndeII[dam-]    alwI[dam-]
            nlaIII             maeII                                        dpnI[dam+] mboII[dam-]
            rcaI            tru9I                      hgaI                 dpnII[dam-]        bstYI/xhoII
            bspHI           mseI                    ddeI
5801 ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT
     TATTAGAGTA CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA AGAACTCTAG GAAAAAAAGA

                                                                     sau3AI
      thaI                                                           mboI/ndeII[dam-]
      fnuDII/mvnI                                                    dpnI[dam+]
      bstUI                                                          dpnII[dam-]
      bsh1236I                                                      alwI[dam-]
     hinPI       fnu4HI                           aciI            mspI                                bsrI
     hhaI/cfoI   bbvI                  aciI    nspBII             hpaII      aluI                 maeIII   eco57I
5901 GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC
     CGCGCATTAG ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTCGCCACC AAACAAACGG CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG

           hinPI                       rmaI              haeIII/palI
           hhaI/cfoI                   maeI       bslI   haeI                 scfI    aciI       mnlI
6001 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC
     AAGTCGTCTC GCGTCTATGG TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG CGGATGTATG GAGCGAGACG
```

FIG. 6Q

```
                                                      scrFI                                             aciI
                                                      nciI                                            nspBII
                      fnu4HI                          mspI                         mspI              fnu4HI
              alwNI     bbvI                          hpaII                        hpaII             bbvI
             bsrI   fnu4HI                            dsaV      pleI              bsaWI            hinPI mcrI
        maeIII        bbvI    bsrI                    cauII     hinfI           maeIII             hhaI/cfoI
6101 TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG
     ATTAGGACAA TGGTCACCGA CGACGGTCAC CGCTATTCAG CACAGAATGG CCCAACCTGA GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC

              hgiAI/aspHI
              bsp1286
              bsiHKAI
              bmyI                                                                                 hinPI
              apaLI/snoI                                                                           hhaI/cfoI
              alw44I/snoI   aluI                                  ddeI      scfI                  haeII
6201 AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCATTGAG AAAGCGCCAC GCTTCCCGAA
     TTGCCCCCCA AGCACGTGTG TCGGGTCGAA CCTCGCTTGC TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGTAACTC TTTCGCGGTG CGAAGGGCTT

                                                                                 scrFI
                                                                                 mvaI          scrFI
                                                                                 ecoRII  mvaI
                       mspI                                                      dsaV    ecoRII
                       hpaII                                                     bstNI        dsaV
                       bslI   fnu4HI                             hinPI mnlI      bsaJI         bstNI
            aciI      bsaWI   aciI                               hhaI/cfoI  aluI apyI[dcm+]    apyI[dcm+]
6301 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
     CCCTCTTTCC GCCTGTCCAT AGGCCATTCG CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC CCCCTTTGCG GACCATAGAA ATATCAGGAC

                                                                                                     haeIII/palI
                                                                                                   fnu4HI
                                                                                                   aciI
                                                                                                  thaI bslI
                                                                                                  fnuDII/mvnI
                           taqI                                     nlaIV                         bstUI
               mnlI drdI   hgaI          sfaNI                   aciI                             bsh1236I      nlaIV
6401 TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT
     AGCCCAAAGC GGTGGAGACT GAACTCGCAG CTAAAAACAC TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG TTGCGCCGGA AAAATGCCAA
```

FIG. 6R

```
   haeIII/palI
  haeI
 scrFI
 mvaI bslI
 ecoRII                 nlaIII
 dsaV                  nspI
 bstNI       haeIII/palI  nspHI                        tfiI                                       bsrBI
 apyI[dcm+]    haeI        aflIII                      hinfI                  aciI         aluI    aciI
6501 CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT
     GGACCGGAAA ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA AACTCACTCG ACTATGGCGA

                                                                                          thaI
                                                                                          fnuDII/mvnI
                                                                                          bstUI
                                                                                          bsh1236I
                                                                                         hinPI
                                                                                         hhaI/cfoI
                                                                                        thaI
                                                                                        fnuDII/mvnI
    fnu4HI              fnu4HI                       sapI  hinPI                        bstUI   haeIII/palI
    bbvI                bbvI pleI                    mboII hhaI/cfoI                    bsh1236I            tru9I
   aciI                hinPI  hinfI                  earI/ksp632I               mnlI  bslI     eaeI  tfiI aseI/asnI/vspI
  fnu4HI       mcrI    hhaI/cfoI          mnlI  aciI     haeII             aciI       aciI     cfrI  hinfI mseI
6601 CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG CCGATTCATT
     GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC AGTCACTCGC TCCTTCGCCT TCTCGCGGGT TATGCGTTTG GCGGAGAGGG GCGCGCAACC GGCTAAGTAA

                                                                                             scrFI
                                                                                             mvaI
                                                                                             ecoRII
                                                                                             dsaV
    aluI                                                   tru9I                        nlaIV bstNI
   pvuII                                        hinPI      mseI       maeIII            hgiCI apyI[dcm+]
   nspBII                  bsrI      aciI       hhaI/cfoI aseI/asnI/vspI mnlI           banI bsaJI
6701 AATCCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA
     TTAGGTCGAC CGTGCTGTCC AAAGGGCTGA CCTTTCGCCC GTCACTCGCG TTGCGTTAAT TACACTCAAT GGAGTGAGTA ATCCGTGGGG TCCGAAATGT

                                                                                           tru9I
                                                                                           mseI
                                                                                          aseI/asnI/vspI
            mspI                          aciI                                          xmnI
            hpaII                       bsrBI                     aluI       nlaIII     asp700
6801 CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA
     GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAATT

>length: 6889
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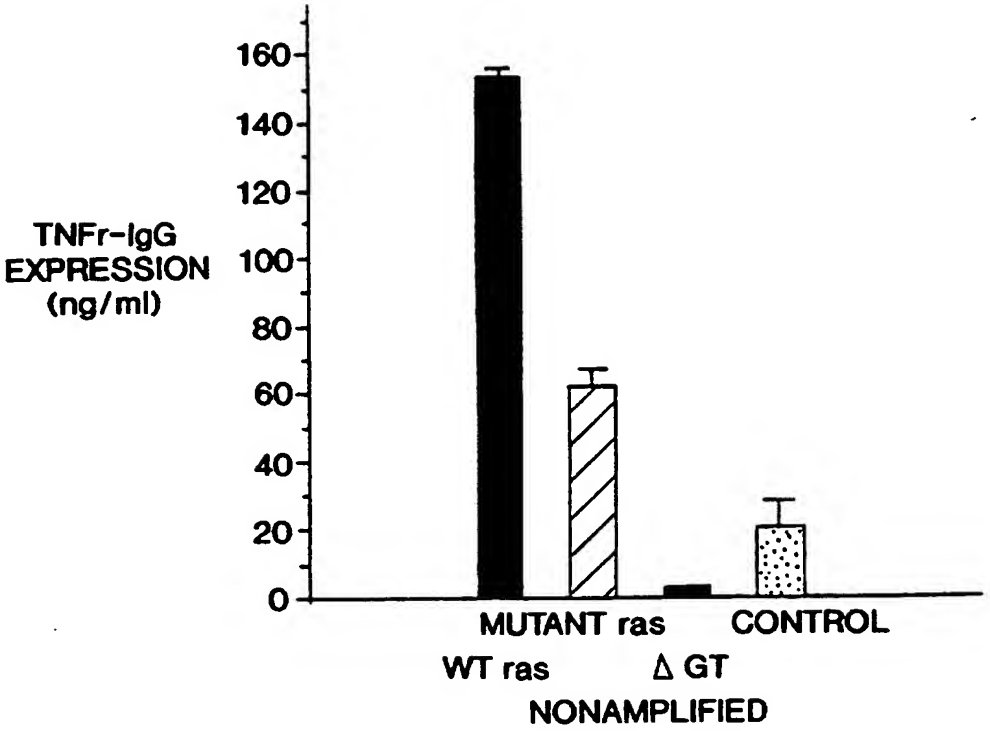
TNFr-IgG EXPRESSION (ng/ml)
160
140
120
100
80
60
40
20
0
MUTANT ras
CONTROL
WT ras
Δ GT
NONAMPLIFIED

FIG. 7A

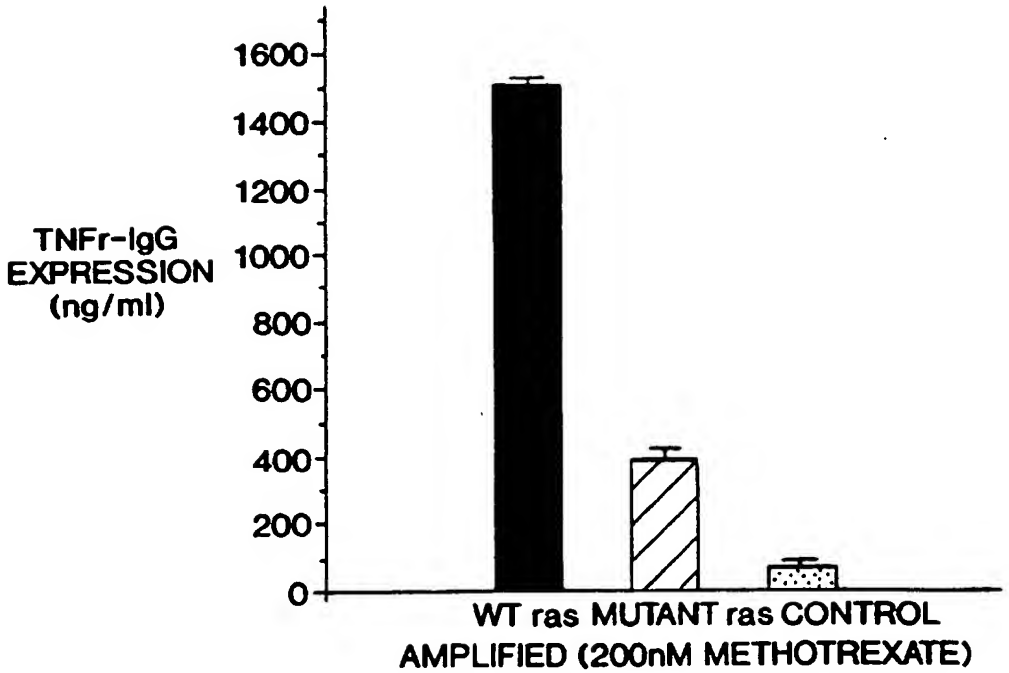
TNFr-IgG EXPRESSION (ng/ml)
1600
1400
1200
1000
800
600
400
200
0
WT ras MUTANT ras CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 7B

FIG. 8

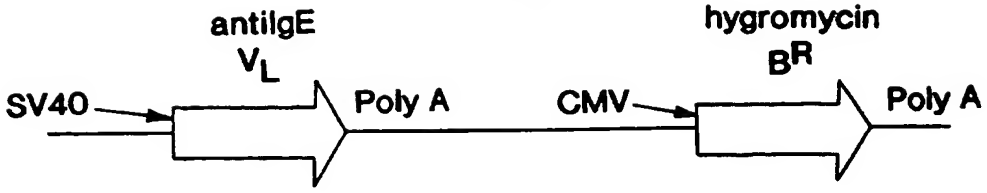
antilgE
V_L
hygromycin
B^R
SV40
Poly A
CMV
Poly A

FIG. 11

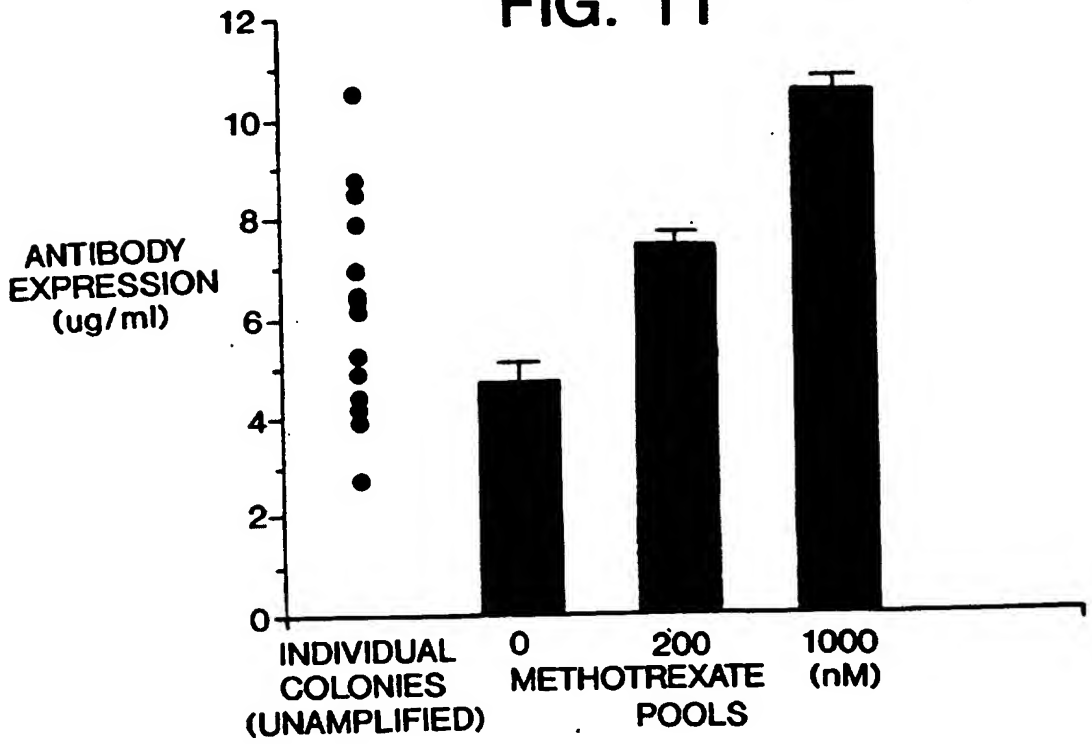
ANTIBODY EXPRESSION (ug/ml)
12
10
8
6
4
2
0
INDIVIDUAL COLONIES (UNAMPLIFIED)
0
200
1000
(nM)
METHOTREXATE POOLS

FIG. 9A

```
     aluI
    sstI
    sacI
    hgiJII
    hgiAI/aspHI
    ecl136II
    bsp1286
    bsiHKAI
    bmyI
    banII                                 aluI                                                          nlaIV
   taqI                          sau3AI pvuII                                                          scrFI
                                 mboI/ndeII[dam-]                                                      mvaI
                                 dpnI[dam+]                                                            ecoRII
                                pvuI/bspCI                                                             dsaV
                              pleI dpnII[dam-]                                                         bstNI
                              hinfI  taqI[dam-]                                                        apyI[dcm+]
                          rmaI    mcrI    nspBII                                                      bsaJI
                          maeI   taqI[dam-]
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT

                                                            nlaIV
          sfaNI                 scrFI        scrFI                                 sfaNI
        ppu10I                  mvaI             mvaI                                      ppu10I
        nsiI/avaIII             ecoRII       ecoRII                              nsiI/avaIII
       nlaIII                   dsaV         dsaV                               nlaIII
      sphI                      bstNI            bstNI                                       sphI
      nspI                      apyI[dcm+]       apyI[dcm+]                                  nspI
      nspHI                    sexAI            bsaJI                                        nspHI
101 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
    CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT

                                                                                   nlaIII
                                                                                  styI
                                                                                  ncoI
                                                 aciI                          bslI dsaI
           aciI           aciI   fokI                      aciI bsrI   aciI     aciI bsaJI
201 GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT
    CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA

                                                                           rmaI
                                                                          styI
                                                                          bsaJI                        haeIII/palI
          fnu4HI                                                          blnI                        mcrI
          bglI                                                            avrII               aluI    eagI/xmaIII/eclXI
          sfiI                                                          haeIII/palI          rmaI     eaeI
         haeIII/palI                                                    stuI                  maeI     cfrI
       mnlI   mnlI      ddeI                                            haeI                 nheI     mspI
     haeIII/palI bsaJI mnlI   aluI                         mnlI         mnlI maeI             aluI     hpaII
    mnlI bsaJI aciI   haeIII/palI                       mnlI
301 TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG
    ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC
```

FIG. 9B

```
                              tfiI
    scrFI                     hinfI
    nciI                   aciI
    mspI                  thaI                                                                    fnu4HI
    hpaII                 fnuDII/mvnI                         aciI                                bbvI
    dsaV                  bstUI                maeII  rsaI                                      nspBII
    cauII                 bsh1236I        maeIII    csp6I  scfI            mnlI          aciI       nlaIII
401 CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT
    GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCACTGCATT CATGGCGGAT ATCTCGCTAT TCTCCTAAAA TAGGGGCGAC GGTAGTACCA

                                                                              haeIII/palI
                                                                             haeI
                                                                           scrFI
                                                                           mvaI     bsrBI
                                                                           ecoRII
                                                                           dsaV
                                                                           bstNI    aciI                  rsaI
                                     pflMI                       bsmAI     apyI[dcm+]        xmnI         csp6I
    taqI           sfaNI             bslI                        bsaI     bsaJI  mnlI  ddeI  asp700      scaI
501 TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC
    AGCTGGTAAC TTGACGTAGC AGCGGCACAG GGTTTTATAC CCCTAACCGT TCTTGCCTCT GGATGGGACC GGAGGCGAGT CCTTGCTCAA GTTCATGAAG

                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                        eco57I                                            dsaV
                       mboII                 tfiI                         bstNI                   tfiI       tru9I
                      earI/ksp632I           hinfI                        apyI[dcm+]              hinfI      mseI
                     mnlI                  alwNI    hphI                 sexAI           ddeI mboII taqI   ahaIII/draI
601 CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA
    GTTTCTTACT GGTGTTGGAG AAGTCACCTT CCATTTGTCT TAGACCACTA ATACCCATCC TTTTGGACCA AGAGGTAAGG ACTCTTCTTA GCTGGAAATT

                                                            sstI
                                                            sacI
                                                            hgiJII
                                                            hgiAI/aspHI
                                                            ecl136II
                                                            bsp1286
                                                            bsiHKAI
             tru9I                                          bmyI                              tru9I
             mseI         ddeI                              banII                            aflII/bfrI
            aseI/asnI/vspI                       bslI   mnlI aluI           bstXI      fokI sfaNI mseI
701 AGGACAGAAT TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA
    TCCTGTCTTA ATTATATCAA GAGTCATCTC TTGAGTTTCT TGGTGGTGCT CCTCGAGTAA AAGAACGGTT TTCAAACCTA CTACGGAATT CTGAATAACT
```

FIG. 9C

```
                                                                                             haeIII/palI
                                                                                            haeI
                                                                        scrFI         scrFI
                                                                        mvaI                mvaI
                                                                        ecoRII        ecoRII
     mspI                                                               dsaV   tfiI   dsaV
      hpaII                                                             bstNI   nlaIII      bstNI     ddeI pleI
     bsaWI                accI nlaIII         mnlI                      apyI[dcm+]  hinfI   apyI[dcm+]     hinfI
 801 ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT
     TGTTGGCCTT AACCGTTCAT TTCATCTGTA CCAAACCTAT CAGCCTCCGT CAAGACAAAT GGTCCTTCGG TACTTAGTTG GTCCGGTGGA ATCTGAGAAA

                                                                                                 hgaI
                                                                                                hinlI/acyI
                                                                                                ahaII/bsaHI
                                                                                              scrFI
         nlaIII                                                                               mvaI     mnlI
       sau3AI                                                                                 ecoRII
       mboI/ndeII[dam-]                                                                       dsaV
       dpnI[dam+]               maeII                                                         bstNI    ecoNI
       dpnII[dam-]             aflIII                                                         apyI[dcm+]      mnlI
     maeIII alwI[dam-]  apoI  maeIII                                             mnlI             bsaJI    bslI ddeI
 901 GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT TGATTTGGGG AAATATAAAC CTCTCCCAGA ATACCCAGGC GTCCTCTCTG
     CACTGTTCCT AGTACGTCCT TAAACTTTCA CTGTGCAAAA AGGGTCTTTA ACTAAACCCC TTTATATTTG GAGAGGGTCT TATGGGTCCG CAGGAGAGAC

       scrFI
       mvaI
       ecoRII
       dsaV
       bstNI
       apyI[dcm+]
     sau96I
     avaII                                                             sfaNI
     asuI  mnlI      sfaNI           accI    mboII                       mboII                       mnlI     aluI
1001 AGGTCCAGGA GGAAAAAGGC ATCAAGTATA AGTTTGAAGT CTACGAGAAG AAAGACTAAC AGGAAGATGC TTTCAAGTTC TCTGCTCCCC TCCTAAAGCT
     TCCAGGTCCT CCTTTTTCCG TAGTTCATAT TCAAACTTCA GATGCTCTTC TTTCTGATTG TCCTTCTACG AAAGTTCAAG AGACGAGGGG AGGATTTCGA
                                                 styI
                                                 bsaJI
                                            sau3AI
                  nlaIII                    mboI/ndeII[dam-]
                 styI                       dpnI[dam+]
                 ncoI                       dpnII[dam-]             aluI     tru9I
     ppu10I      dsaI                       alwI[dam-]             fnu4HI    mseI
     nsiI/avaIII bsaJI                     bstYI/xhoII             bbvI     aseI/asnI/vspI
1101 ATGCATTTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCCTTGG CTTCGTTAGA ACGCAGCTAC AATTAATACA TAACCTTATG TATCATACAC
     TACGTAAAAA TATTCTGGTA CCCTGAAAAC GACCGAAATC TAGGGGAACC GAAGCAATCT TGCGTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG
```

FIG. 9D

```
                                                                     sau96I
                                                                     avaII
                                                                     asuI
                                                                  scrFI
                                                                  mvaI
                                                                  ecoRII
                                                                  dsaV
                                                                  bstNI                                ecoRI
            maeIII                                                apyI[dcm+]       mnlI        taqI    apoI
           hphI    scfI        fokI                         bslI  bsaJI            bsaJI      claI/bsp106
1201 ATACGATTTA GGTGACACTA TAGATAACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT
     TATGCTAAAT CCACTGTGAT ATCTATTGTA GGTGAAACGG AAAGAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGATA GCTAACTTAA

                                                                                                   scrFI
        nlaIII                                                                                     mvaI
       styI                                                                                        ecoRII
       pflMI                                                                                       dsaV
       ncoI                                                                                        bstNI fnu4HI
       dsaI                                                       rsaI              aluI           apyI[dcm+]
       bslI fokI                       rmaI             gsuI/bpmI               pvuII       pleI       haeI    bbvI
       bsaJI       nlaIII  fokI        maeI            bsrI    csp6I            nspBII      hinfI  aciI haeIII/palI
1301 CCACCATGGG ATGGTCATGT ATCATCCTTT TTCTAGTAGC AACTGCAACT GGAGTACATT CAGAAGTTCA GCTGGTGGAG TCTGGCGGTG GCCTGGTGCA
     GGTGGTACCC TACCAGTACA TAGTAGGAAA AAGATCATCG TTGACGTTGA CCTCATGTAA GTCTTCAAGT CGACCACCTC AGACCGCCAC CGGACCACGT
```

FIG. 9E

```
                                                                                                            scrFI
                                                                                                            nciI
                                                                                                            mspI
                                                                                                            hpaII
                                                                                                            dsaV
                                                                                                            cauII
                                                                                                            bslI
                                                                                                           xmaI/pspAI
                                                                                                           smaI
                                                                                                           scrFI
                                                                                                           nciI
                                                                                                           dsaV
                                                                                                           cauII        scrFI
      hgiJII                                                                                            sau96I          mvaI
      bsp1286                                                                                  sau3AI   nlaIV           ecoRII
      bmyI                                                               mspI                  mboI/ndeII[dam-]         dsaV
 scrFI                                                              hpaII                 dpnI[dam+]  bslI        bstNI
 mvaI banII                                                              mroI                  dpnII[dam-]  bsaJI         bslI
 ecoRII                                                            bspMII                nlaIV      haeIII/palI     apyI[dcm+]
 dsaV                                                              bspEI                 bstYI/xhoII    avaI       sau96I
 bstNI                                                                   bsaWI                 bamHI     eco0109I/draII haeIII/palI
 bsaJI                                                                 mnlI                    alwI[dam-] asuI         asuI
 apyI[dcm+]                    alwNI                               hphI  accIII     aluI   bsrI alwI[dam-]  bsaJI     eco0109I/draII
1401 GCCAGGGGGC TCACTCCGTT TGTCCTGTGC AGTTTCTGGC TACTCCATCA CCTCCGGATA TAGCTGGAAC TGGATCCGTC AGGCCCCGGG TAAGGGCCTG
     CGGTCCCCCG AGTGAGGCAA ACAGGACACG TCAAAGACCG ATGAGGTAGT GGAGGCCTAT ATCGACCTTG ACCTAGGCAG TCCGGGGCCC ATTCCCGGAC

                               sau3AI
                               mboI/ndeII[dam-]
                               dpnI[dam+]                                                     thaI
                maeII          dpnII[dam-]                                                    fnuDII/mvnI
           taqI snaBI        alwI[dam-]           hgaI      haeIII/palI                        bstUI  tfiI
          claI/bsp106      mspI                 xmaI       sau96I                             bsh1236I
       sfaNI     bsaAI     hpaII taqI[dam-]     maeI       asuI                           nruI     hinfI
1501 GAATGGGTTG CATCGATTAC GTATGCCGGA TCGACTAACT ATAACCCTAG CGTCAAGGGC CGTATCACTA TAAGTCGCGA CGATTCCAAA AACACATTCT
     CTTACCCAAC GTAGCTAATG CATACGGCCT AGCTGATTGA TATTGGGATC GCAGTTCCCG GCATAGTGAT ATTCAGCGCT GCTAAGGTTT TTGTGTAAGA
```

FIG. 9F

```
                                                                                            scrFI
                                                                                            mvaI
                                                                                            ecoRII
                                                                                            dsaV
                                                                                            bstNI
                                                                                            apyI[dcm+]
                                                                                         hinPI
                                                                                         hhaI/cfoI
                                                            mnlI                        nlaIV
                                                         xhoI                           narI
                                                         paeR7I                         kasI
                                                         avaI                           hinlI/acyI
   scfI                                              hgiAI/aspHI                        hgiCI
   pstI                                              bsp1286                            haeII
   bsgI                            mnlI              bsiHKAI    fnu4HI                  banI
 bspMI                           ddeI drdI           bmyI taqI bbvI                     ahaII/bsaHI
1601 ACCTGCAGAT GAACAGCCTG CGTGCTGAGG ACACTGCCGT CTATTATTGT GCTCGAGGCA GCCACTATTT CGGCGCCTGG CACTTCGCCG TGTGGGGTCA
     TGGACGTCTA CTTGTCGGAC GCACGACTCC TGTGACGGCA GATAATAACA CGAGCTCCGT CGGTGATAAA GCCGCGGACC GTGAAGCGGC ACACCCCAGT

                                         sau96I
                                         haeIII/palI
                                        sau96I
                                        nlaIV                                                                      scrFI
                                        hgiJII                   nlaIV                                             mvaI
                                        bsp1286                  hgiCI                                             ecoRII
       scrFI                       bsp120I                  banI                                                   dsaV
       mvaI                             bmyI                  scrFI                                          bstNI
       ecoRII                      banII               mvaI                                                  bsaJI
       dsaV                        asuI                ecoRII                                              sau96I
       bstNI hphI                                                                                       haeIII/palI
       apyI[dcm+] bsmAI                 apaI          mboII  dsaV            hgiAI/aspHI                   asuI
      bsaJI maeIII    mnlI  haeIII/palI eco0109I/draII       bstNI                bsp1286                    fnu4HI
   nlaIV  bstEII esp3I bsaJI mnlI  styI asuI           bpuAI apyI[dcm+]  mnlI     bsiHKAI           bsp1286 aciI apyI[dcm+]
                                    bsaJI               bbsI  bsaJI   mnlI       bmyI mnlI         bmyI nspBII bsaJI
1701 AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
```

FIG. 9G

```
        scrFI                                                  hinPI
        mvaI                                                        nlaIV
        ecoRII                                                 narI
        ecoNI                                                       kasI          hgiAI/aspHI
        dsaV                     hphI                          hinlI/acyI     bsp1286     mspI
        bstNI                       mspI                            hgiCI          bsiHKAI    hpaII
        bslI                        hpaII                           haeII          bmyI      scrFI
        apyI[dcm+]                 cfr10I                           banI       fnu4HI        nciI
     fnu4HI                        bsaWI  tth111I/aspI              ahaII/bsaHI aciI  apaLI/snoI dsaV
     bbvI                   bslI   ageI maeIII                   ddeI hhaI/cfoI nspBII alw44I/snoI cauII     scfI
1801 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA

                                                               nlaIV
     ddeI pleI                                          fnu4HI hgiCI
    mnlI  hinfI                                   mnlI  bbvI   banI                   tfiI
    eco81I               mnlI  fnu4HI  maeIII bsp1286 rmaI     bsp1286                hinfI                 styI
    bsu36I/mstII/sauI ddeI bbvI    hphI    bmyI    maeI aluI   bmyI               maeII                     bsaJI
1901 CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACTGTGCC CTCTAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC
     GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGACACGG GAGATCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG

                                                                                         eam1105I
                                                                                         sau96I
                                                                                  scrFI
                                                                                  mvaI    avaII
                                                                                  ecoRII
                             hgiJII                                               dsaV
                             bsp1286                       nlaIII                 bstNI   asuI        mboII mboII
                             bmyI                         nspI         bsp1286    bsaJI  nlaIV      bpuAI earI/ksp632I
                             banII        maeIII          nspHI        bmyI  alwNI apyI[dcm+]       bbsI mnlI
2001 CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC
     GTTCCACCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CGGGTGGCAC GGGTCGTGGA CTTGAGGACC CCCCTGGCAG TCAGAAGGAG
```

FIG. 9H

```
                                             sau96I
                                             nlaIV
                                             avaII
                                           mspI
                                          scrFI
                                          nciI
                                    sau3AI hpaII
                                    mboI/ndeII[dam-]
                                    dpnI[dam+]                nlaIII
                                 nlaIII   dsaV        mnlI   nspI                            drdI  mnlI
                                rcaI      cauII     ddeI maeIII                           mboII   ddeI
                   styI         bspHI[dam-]  asuI  eco81I    nspHI                        bpuAI   eco81I
                   bsaJI      mnlI  dpnII[dam-]    bsu36I/mstII/sauI          maeII       bbsI   bsu36I/mstII/sauI
2101 TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA
     AAGGGGGGTT TTGGGTTCCT GTGGGAGTAC TAGAGGGCCT GGGGACTCCA GTGTACGCAC CACCACCTGC ACTCGGTGCT TCTGGGACTC CAGTTCAAGT

                                                       aciI
                                                      thaI
                                                      fnuDII/mvnI
                                                      bstUI
                                                      bsh1236I
                                                     sacII/sstII
                                                     nspBII
                                                     kspI
           maeII                                     dsaI                            rsaI
         rsaI                                        bsaJI                           csp6I
         csp6I                                       aciI           rsaI       maeII                  mnlI      ecoNI
     bsrI bsaAI          mnlI                       fnu4HI  mnlI    csp6I     bsaAI              hgaI   hphI   bslI
2201 ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT
     TGACCATGCA CCTGCCGCAC CTCCACGTAT TACGGTTCTG TTTCGGCGCC CTCCTCGTCA TGTTGTCGTG CATGGCACAC CAGTCGCAGG AGTGGCAGGA

        scrFI
        mvaI bsrI
        ecoRII
        dsaV
        bstNI                     rsaI           bsmAI                                                       fnu4HI
        apyI[dcm+]                csp6I        bsaI            mnlI           taqI                           bbvI
2301 GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG
     CGTGGTCCTG ACCGACTTAC CGTTCCTCAT GTTCACGTTC CAGAGGTTGT TTCGGGAGGG TCGGGGGTAG CTCTTTTGGT AGAGGTTTCG GTTTCCCGTC
```

FIG. 9I

```
                                                  scrFI
                                                  nciI
                                                  mspI
                                                  hpaII
                                                  dsaV
                                                  cauII
                                                 xmaI/pspAI
                                                 smaI
                                                 scrFI                 scrFI
                                                 nciI                  mvaI             scrFI
                                                 dsaV                  ecoRII      mvaI
                                                 cauII                 dsaV        ecoRII
                     rsaI              fokI   mboII                    bstNI             dsaV
                     csp6I          bslI bsaJI                         apyI[dcm+]        bstNI
     avaI           bsp1407I       bslI  avaI earI/ksp632I           sexAI         bspMI apyI[dcm+]
2401 CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG AAGAGATGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG
     GGGGCTCTTG GTGTCCACAT GTGGGACGGG GGTAGGGCCC TTCTCTACTG GTTCTTGGTC CAGTCGGACT GGACGGACCA GTTTCCGAAG ATAGGGTCGC

          dsaI                  mspI
          bslI              fnu4HI                                    pleI                 mnlI
          bsaJI             bbvI hpaII                 mnlI           hinfI      nlaIV   mboII   scfI    aluI
2501 ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA
     TGTAGCGGCA CCTCACCCTC TCGTTACCCG TCGGCCTCTT GTTGATGTTC TGGTGCGGAG GGCACGACCT GAGGCTGCCG AGGAAGAAGG AGATGTCGTT

                                                   bpuAI
                                                   bbsI               nlaIII
         dsaI                                    maeII              ppu10I                              sapI
         bsaJI                   fnu4HI        xmnI mboII           nsiI/avaIII                         mboII   mnlI
     hphI             bspMI      bbvI          asp700      nlaIII  sfaNI   mnlI                         earI/ksp632I bslI
2601 GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC
     CGAGTGGCAC CTGTTCTCGT CCACCGTCGT CCCCTTGCAG AAGAGTACGA GGCACTACGT ACTCCGAGAC GTGTTGGTGA TGTGCGTCTT CTCGGAGAGG

                                                                      styI
          scrFI                                 taqI               aciI
          nciI                                 salI               fnu4HI   sau96I
          mspI                        rmaI  hincII/hindII        sfiI ncoI haeIII/palI
          hpaII                       maeI  accI     scfI         haeIII/palI
          dsaV                    sau96I  pleI       pstI       eaeI  dsaI asuI                 aluI
          cauII                   haeIII/palI        bsgI    aluI cfrI  bsaJI                 fnu4HI
      bsmAI                       asuI    hinfI bspMI       hindIII bglI nlaIII               bbvI          maeIII
2701 CTGTCTCCGG GTAAATGAGT GCGACGGCCC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT
     GACAGAGGCC CATTTACTCA CGCTGCCGGG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA
```

FIG. 9J

```
                                                                                                                taqI[dam-]
                                                                                                               claI/bsp106[dam-]
                                                                                                              sau3AI
                                                                                                              mboI/ndeII[dam-]
                                                                                                              dpnI[dam+]
                                                                    rmaI                                      dpnII[dam-]
             sfaNI    apoI                                   bsmI   maeI                               nlaIII alwI[dam-]
2801 AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT
     TTTCGTTATC GTAGTGTTTA AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA

               tru9I                                                          rsaI
           xmnI                                                               csp6I
      sau3AI  aseI/asnI/vspI    styI                                         nlaIV
      mboI/ndeII[dam-]          ncoI                                         kpnI
      dpnI[dam+]          fnu4HI   haeI                                      hgiCI
      dpnII[dam-]         bbvI  dsaI haeIII/palI                             banI                         aluI
     pvuI/bspCI mseI   hinPI    bsaJI                                        asp718     mnlI            pvuII
     mcrI   asp700     hhaI/cfoI nlaIII         mnlI      mnlI               acc65I  ddeI aciI          nspBII
2901 CGATCGGGAA TTAATTCGGC GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA
     GCTAGCCCTT AATTAAGCCG CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT

                                   nlaIV
                               scrFI                                                           scrFI
                               mvaI                                        ppu10I                   mvaI
                               ecoRII                                nsiI/avaIII               ecoRII
                               dsaV                                 nlaIII                     dsaV
                               bstNI                                    sphI                        bstNI
                               apyI[dcm+]                               nspI sfaNI                  apyI[dcm+]
                              bsaJI                                     nspHI                     sexAI          bsaJI
3001 TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC
     ACACACAGTC AATCCCACAC CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG

        nlaIV                           sfaNI
     scrFI                     ppu10I
     mvaI                            nsiI/avaIII
     ecoRII                   nlaIII
     dsaV                    sphI
     bstNI                           nspI                                            aciI
     apyI[dcm+]                      nspHI                       aciI         aciI  fokI          aciI
3101 CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC
     GGTCCGAGGG GTCGTCCGTC TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG
```

FIG. 9K

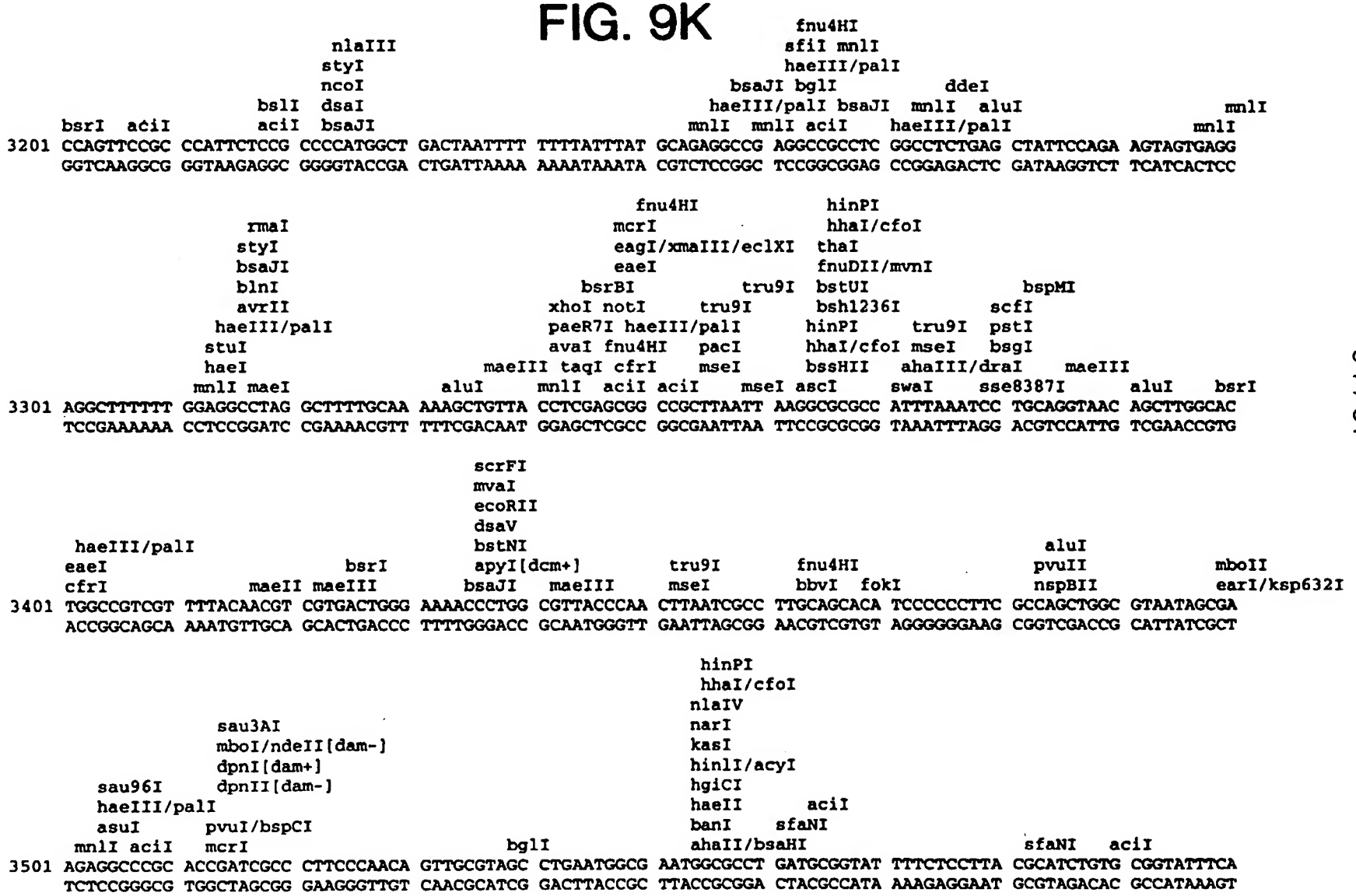
fnu4HI
nlaIII
sfiI mnlI
styI
haeIII/palI
ncoI
bsaJI bglI ddeI
bslI dsaI
haeIII/palI bsaJI mnlI aluI mnlI
bsrI aciI aciI bsaJI
mnlI mnlI aciI haeIII/palI mnlI
3201 CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG
GGTCAAGGCG GGTAAGAGGC GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC
fnu4HI
hinPI
rmaI
mcrI
hhaI/cfoI
styI
eagI/xmaIII/eclXI thaI
bsaJI
eaeI
fnuDII/mvnI
blnI
bsrBI tru9I bstUI bspMI
avrII
xhoI notI tru9I bsh1236I scfI
haeIII/palI
paeR7I haeIII/palI hinPI tru9I pstI
stuI
avaI fnu4HI pacI hhaI/cfoI mseI bsgI
haeI
maeIII taqI cfrI mseI bssHII ahaIII/draI maeIII
mnlI maeI
aluI mnlI aciI aciI mseI ascI swaI sse8387I aluI bsrI
3301 AGGCTTTTTT GGAGGCCTAG GCTTTTGCAA AAAGCTGTTA CCTCGAGCGG CCGCTTAATT AAGGCGCGCC ATTTAAATCC TGCAGGTAAC AGCTTGGCAC
TCCGAAAAAA CCTCCGGATC CGAAAACGTT TTTCGACAAT GGAGCTCGCC GGCGAATTAA TTCCGCGCGG TAAATTTAGG ACGTCCATTG TCGAACCGTG
scrFI
mvaI
ecoRII
dsaV
haeIII/palI
bstNI
aluI
eaeI
bsrI
apyI[dcm+]
tru9I
fnu4HI
pvuII
mboII
cfrI
maeII maeIII
bsaJI maeIII mseI
bbvI fokI
nspBII
earI/ksp632I
3401 TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG CGTTACCCAA CTTAATCGCC TTGCAGCACA TCCCCCCTTC GCCAGCTGGC GTAATAGCGA
ACCGGCAGCA AAATGTTGCA GCACTGACCC TTTTGGGACC GCAATGGGTT GAATTAGCGG AACGTCGTGT AGGGGGGAAG CGGTCGACCG CATTATCGCT
hinPI
hhaI/cfoI
nlaIV
sau3AI
narI
mboI/ndeII[dam-]
kasI
dpnI[dam+]
hinlI/acyI
sau96I
dpnII[dam-]
hgiCI
haeIII/palI
haeII aciI
asuI
pvuI/bspCI
banI sfaNI
mnlI aciI
mcrI
bglI
ahaII/bsaHI
sfaNI aciI
3501 AGAGGCCCGC ACCGATCGCC CTTCCCAACA GTTGCGTAGC CTGAATGGCG AATGGCGCCT GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTTCA
TCTCCGGGCG TGGCTAGCGG GAAGGGTTGT CAACGCATCG GACTTACCGC TTACCGCGGA CTACGCCATA AAAGAGGAAT GCGTAGACAC GCCATAAAGT

FIG. 9L

```
                                                          aciI
               hinPI                                    fnu4HI                fnu4HI
               hhaI/cfoI                                aciI                 hinPI
              thaI                                     thaI                  hhaI/cfoI
              fnuDII/mvnI                              fnuDII/mvnI          thaI
              bstUI              hinPI                 bstUI                fnuDII/mvnI
              bsh1236I           hhaI/cfoI hinPI       bsh1236I             bstUI
            rsaI      scfI fnu4HI     tru9I bsh1236I                        bsh1236I    aciI               hinPI
  aciI  maeII   csp6I  bslI  aciI    mseI hhaI/cfoI    maeIII  bbvI  maeIII                               hhaI/cfoI
                                                                                                          haeII
3601 CACCGCATAC GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG
     GTGGCGTATG CAGTTTCGTT GGTATCATGC GCGGGACATC GCCGCGTAAT TCGCGCCGCC CACACCACCA ATGCGCGTCG CACTGGCGAT GTGAACGGTC

       hinPI                                                                              nlaIV
       hhaI/cfoI                                     mspI                                 hgiJII
      haeII                                          hpaII                                bsp1286
    rmaI    bsrBI                                    naeI                                 bmyI
    maeI    aciI          mboII               maeII  cfr10I          aluI                 banII       nlaIV
3701 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC
     GCGGGATCGC GGGCGAGGAA AGCGAAAGAA GGGAAGGAAA GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG CCCCCGAGGG AAATCCCAAG

                nlaIV                                    maeII   haeIII/palI
                hgiCI  taqI                              draIII  sau96I                                   maeII
                banI mnlI                      hphI      bsaAI   asuI                                     drdI
3801 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA
     GCTAAATCAC GAAATGCCGT GGAGCTGGGG TTTTTTGAAC TAAACCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT CTGCCAAAAA GCGGGAAACT

      pleI          tru9I      pleI                                 bslI
      hinfI  maeII  mseI       hinfI          bsrI                  bslI   avaI
3901 CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG CTATTCTTTT GATTTATAAG GGATTTTGCC
     GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCC GATAAGAAAA CTAAATATTC CCTAAAACGG

                                                   thaI                                   hgiAI/aspHI
                                                   fnuDII/mvnI                            bsp1286
                                                   bstUI                   maeII          bsiHKAI
                tru9I                            tru9I                   tru9I            bmyI     ddeI
                mseI                 tru9I       mseI    apoI tru9I      mseI             apaLI/snoI  rsaI
       haeIII/palI          aluI     mseI    apoI    bsh1236I mseI   sspI  psp1406I       alw44I/snoI csp6I
4001 GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTTATGGTG CACTCTCAGT
     CTAAAGCCGG ATAACCAATT TTTTACTCGA CTAAATTGTT TTTAAATTGC GCTTAAAATT GTTTTATAAT TGCAAATGTT AAAATACCAC GTGAGAGTCA
```

FIG. 9M

```
                                                                                                          hinPI
                                                                                                          hhaI/cfoI
                                                                                                          thaI
                                                                             hinPI                        fnuDII/mvnI
          aciI                                          maeIII            fnu4HI                          bstUI
          fnu4HI      tru9I                           maeII bsrI     nlaIII  hhaI/cfoI            nspBII  bsh1236I
       sfaNI          mseI         aciI              bsaAI tth111I/aspI bbvI            aciI      aciI  hgaI
4101 ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC
     TGTTAGACGA GACTACGGCG TATCAATTCG GTTGAGGCGA TAGCGATGCA CTGACCCAGT ACCGACGCGG GGCTGTGGGC GGTTGTGGGC GACTGCGCGG

                                                                                                               thaI
                                                                                                               fnuDII/mvnI
                                                                                                               bstUI
                                                                  scrFI                                        bsh1236I
             mspI                                                 nciI                                        hinPI
             hpaII                                                mspI                                        hhaI/cfoI
            scrFI                                           bsmAI         nspI                                thaI
            nciI     aciI                                  esp3I hpaII fnu4HI                                 fnuDII/mvnI
            dsaV sfaNI                                    bslI    dsaV aluI nspHI                             bstUI
  drdI      cauII fokI                     aluI maeIII            cauII bbvI nlaIII   mnlI    hphI      hphI  bsh1236I
4201 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC
     GACTGCCCGA ACAGACGAGG GCCGTAGGCG AATGTCTGTT CGACACTGGC AGAGGCCCTC GACGTACACA GTCTCCAAAA GTGGCAGTAG TGGCTTTGCG

                         mnlI
                       haeIII/palI                                                            maeII
           mboII      sau96I                                       nlaIII                    hinlI/acyI
           bpuAI      asuI                                 tru9I  rcaI                       ahaII/bsaHI
  mnlI     bbsI      eco0109I/draII                        mseI   bspHI              ddeI aatII
4301 GCGAGGCAGT ATTCTTGAAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT GTCATGATAA TAATGGTTTC TTAGACGTCA GGTGGCACTT
     CGCTCCGTCA TAAGAACTTC TGCTTTCCCG GAGCACTATG CGGATAAAAA TATCCAATTA CAGTACTATT ATTACCAAAG AATCTGCAGT CCACCGTGAA

             nlaIV
            aciI
           thaI
           fnuDII/mvnI
           bstUI                                                        rcaI
           bsh1236I                                                     bspHI
          hinPI                                                   bsrBI    bsmAI
          hhaI/cfoI                                               aciI nlaIII                        sspI
4401 TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
     AAGCCCCTTT ACACGCGCCT TGGGGATAAA CAAATAAAAA GATTTATGTA AGTTTATACA TAGGCGAGTA CTCTGTTATT GGGACTATTT ACGAAGTTAT

          mboII                                                   fnu4HI
          earI/ksp632I                                            aciI                    hphI
4501 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG
     TATAACTTTT TCCTTCTCAT ACTCATAAGT TGTAAAGGCA CAGCGGGAAT AAGGGAAAAA ACGCCGTAAA ACGGAAGGAC AAAAACGAGT GGGTCTTTGC
```

FIG. 9N

```
                                          hgiAI/aspHI
                                          bsp1286
                              sau3AI      bsiHKAI                             sau3AI  nspBII    sau3AI
                              mboI/ndeII[dam-]                                mboI/ndeII[dam-]  mboI/ndeII[dam-]
                              dpnI[dam+]  bmyI                                dpnI[dam+]        dpnI[dam+]
                              dpnII[dam-]                                   bstYI/xhoII         dpnII[dam-]
                         eco57I           apaLI/snoI                     bsrI dpnII[dam-]       alwI[dam-]
  hphI              sfaNI  mboII[dam-]    alw44I/snoI  maeIII taqI      alwI[dam-]   aciI  bstYI/xhoII             mboII
4601 CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG
     GACCACTTTC ATTTTCTACG ACTTCTAGTC AACCCACGTG CTCACCCAAT GTAGCTTGAC CTAGAGTTGT CGCCATTCTA GGAACTCTCA AAAGCGGGGC

                                                                                 scrFI
                                                                                 nciI
                                                         aciI                    mspI
                                                        thaI                     hpaII
                                                        fnuDII/mvnI              dsaV
   maeII                 hgiAI/aspHI                    bstUI                    cauII
  psp1406I               bsp1286   tru9I                bsh1236I             hinlI/acyI
  xmnI                   bsiHKAI   mseI                 hinPI                hgaI                          aciI
  asp700                 bmyI    ahaIII/draI            hhaI/cfoI            ahaII/bsaHI    bcgI   mcrI   fnu4HI
4701 AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTGATGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
     TTCTTGCAAA AGGTTACTAC TCGTGAAAAT TTCAAGACGA TACACCGCGC CATAATAGGG CACTACTGCG GCCCGTTCTC GTTGAGCCAG CGGCGTATGT

                              rsaI
                              csp6I   bsrI                                                     fnu4HI
    ddeI                     schI hphI   maeIII      sfaNI     fokI    nlaIII                  bbvI       nlaIII
4801 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG
     GATAAGAGTC TTACTGAACC AACTCATGAG TGGTCAGTGT CTTTTCGTAG AATGCCTACC GTACTGTCAT TCTCTTAATA CGTCACGACG GTATTGGTAC

                                                 sau96I
                                                 avaII                                          maeIII
                                          sau3AI asuI                                         nlaIII
               haeIII/palI                mboI/ndeII[dam-]                                   sau3AI
              eaeI                        dpnI[dam+]                                         mboI/ndeII[dam-]  sau3AI
              cfrI                        dpnII[dam-]                                        dpnI[dam+]        mboI/ndeII[dam-]
             fnu4HI                      pvuI/bspCI                                          dpnII[dam-]       dpnI[dam+]
             aciI                        mcrI   mnlI         aluI  aciI             nlaIII alwI[dam-]          dpnII[dam-]
4901 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG
     TCACTATTGT GACGCCGGTT GAATGAAGAC TGTTGCTAGC CTCCTGGCTT CCTCGATTGG CGAAAAAACG TGTTGTACCC CCTAGTACAT TGAGCGGAAC
```

FIG. 9O

```
                                                                                            hinPI
         mspI                                                                               mstI
          hpaII                                                                             aviII/fspI     bsrI
         bsaWI                                                      fnu4HI             maeII hhaI/cfoI   tru9I
      nlaIV     aluI                               maeIII      sfaNI    bbvI              psp1406I         mseI
5001 ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CAGCAGCAAT GGCAACAACG TTGCGCAAAC TATTAACTGG
     TAGCAACCCT TGGCCTCGAC TTACTTCGGT ATGGTTTGCT GCTCGCACTG TGGTGCTACG GTCGTCGTTA CCGTTGTTGC AACGCGTTTG ATAATTGACC

                   mspI
                    hpaII                                                                     bglI
                   scrFI                                                                      sau96I
             aluI  nciI        tru9I      fokI                        sau96I                  haeIII/palI
            rmaI   dsaV        mseI    bsrI        aciI               avaII          hinPI   asuI    mspI
            maeI   cauII       aseI/asnI/vspI   mnlI                  asuI           hhaI/cfoI       hpaII
5101 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG
     GCTTGATGAA TGAGATCGAA GGGCCGTTGT TAATTATCTG ACCTACCTCC GCCTATTTCA ACGTCCTGGT GAAGACGCGA GCCGGGAAGG CCGACCGACC

                    mspI                thaI
                     hpaII              fnuDII/mvnI              haeIII/palI
                    cfr10I              bstUI                    sau96I
               nlaIV  hphI          bsmAI aciI        fnu4HI  nlaIV
             gsuI/bpmI             bsaI bsh1236I      bbvI bsrI asuI             mnlI
5201 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA
     AAATAACGAC TATTTAGACC TCGGCCACTC GCACCCAGAG CGCCATAGTA ACGTCGTGAC CCCGGTCTAC CATTCGGGAG GGCATAGCAT CAATAGATGT

                                                   ddeI
                                               sau3AI        nlaIV
          pleI                                 mboI/ndeII[dam-]  mnlI
          hinfI                                dpnI[dam+]     hgiCI          tru9I
      eam1105I                 fokI            dpnII[dam-]    banI           mseI       maeIII
5301 CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC
     GCTGCCCCTC AGTCCGTTGA TACCTACTTG CTTTATCTGT CTAGCGACTC TATCCACGGA GTGACTAATT CGTAACCATT GACAGTCTGG TTCAAATGAG

                                                       rmaI       sau3AI
                                                   sau3AI hphI  mboI/ndeII[dam-]
                                                   mboI/ndeII[dam-]
                                                   dpnI[dam+]   dpnI[dam+]
                                             tru9I  dpnII[dam-]  dpnII[dam-]
                tru9I                      ahaIII/draI maeI     alwI[dam-]          nlaIII              maeII
                mseI               tru9I      bstYI/xhoII  bstYI/xhoII             rcaI               tru9I
              ahaIII/draI          mseI mseI  alwI[dam-] mboII[dam-]              bspHI              mseI
5401 ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG
     TATATATGAA ATCTAACTAA ATTTTGAAGT AAAAATTAAA TTTTCCTAGA TCCACTTCTA GGAAAAACTA TTAGAGTACT GGTTTTAGGG AATTGCACTC
```

FIG. 9P

```
                                                         sau3AI
                                                         mboI/ndeII[dam-]
                                                         dpnI[dam+]  sau3AI          thaI
                                                         dpnII[dam-] mboI/ndeII[dam-]
                                                        bstYI/xhoII  dpnI[dam+]      fnuDII/mvnI
                                                 sau3AI  alwI[dam-]  dpnII[dam-]     bstUI
                                                 mboI/ndeII[dam-]    alwI[dam-]      bsh1236I
                             hgaI                dpnI[dam+] mboII[dam-]             hinPI       fnu4HI
                         ddeI                    dpnII[dam-]        bstYI/xhoII     hhaI/cfoI   bbvI
5501 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC
     AAAAGCAAGG TGACTCGCAG TCTGGGGCAT CTTTTCTAGT TTCCTAGAAG AACTCTAGGA AAAAAAGACG CGCATTAGAC GACGAACGTT TGTTTTTTTG

                                        sau3AI
                                        mboI/ndeII[dam-]
                                        dpnI[dam+]
                                        dpnII[dam-]
                                       alwI[dam-]
                         aciI        mspI                                    bsrI           hinPI
        aciI   nspBII                hpaII       aluI                    maeIII    eco57I   hhaI/cfoI
5601 CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT
     GTGGCGATGG TCGCCACCAA ACAAACGGCC TAGTTCTCGA TGGTTGAGAA AAAGGCTTCC ATTGACCGAA GTCGTCTCGC GTCTATGGTT TATGACAGGA

                                                                                                       fnu4HI
                                                                                                alwNI  bbvI
      rmaI                 haeIII/palI                                                        bsrI  fnu4HI
      maeI       bslI     haeI               scfI     aciI        mnlI               maeIII    bbvI     bsrI
5701 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
     AGATCACATC GGCATCAATC CGGTGGTGAA GTTCTTGAGA CATCGTGGCG GATGTATGGA GCGAGACGAT TAGGACAATG GTCACCGACG ACGGTCACCG

                  scrFI                                       aciI                        hgiAI/aspHI
                  nciI                                      nspBII                        bsp1286
                  mspI                         mspI        fnu4HI                         bsiHKAI
                  hpaII                        hpaII       bbvI                           bmyI
                  dsaV     pleI               bsaWI       hinPI mcrI                      apaLI/snoI
                  cauII    hinfI           maeIII         hhaI/cfoI                       alw44I/snoI   aluI
5801 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG
     CTATTCAGCA CAGAATGGCC CAACCTGAGT TCTGCTATCA ATGGCCTATT CCGCGTCGCC AGCCCGACTT GCCCCCCAAG CACGTGTGTC GGGTCGAACC
```

FIG. 9Q

```
                                                                                                      mspI
                                                             hinPI                                    hpaII
                                                             hhaI/cfoI                                bslI    fnu4HI
                           ddeI     scfI                    haeII                        aciI        bsaWI    aciI
5901 AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG
     TCGCTTGCTG GATGTGGCTT GACTCTATGG ATGTCGCACT CGTAACTCTT TCGCGGTGCG AAGGGCTTCC CTCTTTCCGC CTGTCCATAG GCCATTCGCC

                                          scrFI
                                          mvaI          scrFI
                                          ecoRII  mvaI
                                          dsaV    ecoRII
                                          bstNI         dsaV
                        hinPI  mnlI       bsaJI         bstNI                                                 taqI
                        hhaI/cfoI   aluI  apyI[dcm+]    apyI[dcm+]                                  mnlI drdI   hgaI
6001 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA
     GTCCCAGCCT TGTCCTCTCG CGTGCTCCCT CGAAGGTCCC CCTTTGCGGA CCATAGAAAT ATCAGGACAG CCCAAAGCGG TGGAGACTGA ACTCGCAGCT

                                                                                   haeIII/palI
                                                               haeIII/palI     scrFI
                                                             fnu4HI            mvaI
                                                             aciI              ecoRII
                                                            thaI bslI          dsaV                    nlaIII
                                                            fnuDII/mvnI        bstNI bslI                   nspI
                                nlaIV                       bstUI              apyI[dcm+]     haeIII/palI   nspHI
            sfaNI             aciI                          bsh1236I       nlaIV  haeI        haeI          aflIII
6101 TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT
     AAAAACACTA CGAGCAGTCC CCCCGCCTCG GATACCTTTT TGCGGTCGTT GCGCCGGAAA AATGCCAAGG ACCGGAAAAC GACCGGAAAA CGAGTGTACA

                                                                                    fnu4HI              fnu4HI
                                                                                    bbvI                bbvI pleI
                           tfiI                                              bsrBI  aciI             hinPI   hinfI
                           hinfI                   aciI          aluI        aciI fnu4HI        mcrI hhaI/cfoI
6201 TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC
     AGAAAGGACG CAATAGGGGA CTAAGACACC TATTGGCATA ATGGCGGAAA CTCACTCGAC TATGGCGAGC GGCGTCGGCT TGCTGGCTCG CGTCGCTCAG
```

FIG. 9R

```
                                                           thaI
                                                           fnuDII/mvnI
                                                           bstUI
                                                           bsh1236I
                                                         hinPI
                                                         hhaI/cfoI
                                                        thaI
                                                        fnuDII/mvnI
                   sapI  hinPI                          bstUI                tru9I   aluI
                   mboII hhaI/cfoI                      bsh1236I haeIII/palI        pvuII
                   earI/ksp632I               mnlI     bslI     eaeI  tfiI aseI/asnI/vspI
        mnlI   aciI     haeII            aciI           aciI     cfrI  hinfI mseI   nspBII           bsrI
6301 AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG
     TCACTCGCTC CTTCGCCTTC TCGCGGGTTA TGCGTTTGGC GGAGAGGGGC GCGCAACCGG CTAAGTAATT AGGTCGACCG TGCTGTCCAA AGGGCTGACC

                                                                 scrFI
                                                                 mvaI
                                                                 ecoRII
                                                                 dsaV
                                 tru9I                      nlaIV bstNI
                      hinPI      mseI        maeIII         hgiCI apyI[dcm+]             mspI
        aciI          hhaI/cfoI aseI/asnI/vspI mnlI         banI bsaJI                   hpaII
6401 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
     TTTCGCCCGT CACTCGCGTT GCGTTAATTA CACTCAATGG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT

                                                              tru9I
                                                              mseI
                                                             aseI/asnI/vspI
         aciI                                                xmnI
       bsrBI                            aluI       nlaIII    asp700
6501 TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GAATTAA
     AACACTCGCC TATTGTTAAA GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAATT

>length: 6557
```

FIG. 10A

```
      aluI
     sstI
     sacI
     hgiJII
     hgiAI/aspHI
     ecl136II
     bsp1286
     bsiHKAI
     bmyI                         rmaI    tru9I                                                        thaI
     banII                        maeI    mseI                                                         fnuDII/mvnJ
    taqI                         speI    aseI/asnI/vspI                            bslI               bstUI
                                                                                                       bsh1236I
                                                                                                      aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                 scrFI
                 mvaI
                 ecoRII
                 dsaV
               aciI
             bglI bstNI                                   maeII
            sau96I                                       hinlI/acyI
            haeIII/palI    aciI                          ahaII/bsaHI
            asuI  apyI[dcm+]          aciI               aatII      maeII       maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

      maeII                                                                                            maeII
     hinlI/acyI                                                                                       hinlI/acyI
     ahaII/bsaHI                              rsaI                    rsaI                            ahaII/bsaHI
     aatII                          bglI      csp6I         ndeI      csp6I                           aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

            scrFI
            mvaI
            ecoRII
         aciI                                                                                             nlaIII
       bglI  dsaV                                                                                 styI
      sau96I bstNI       rsaI                                          maeII                              ncoI
      haeIII/palI        csp6I                            rsaI     snaBI                             dsaI hphI  aciI
      asuI   apyI[dcm+] bsrI  nlaIII                      csp6I    bsaAI                             bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 10B

```
                                                                        maeII
                                                                       hinlI/acyI                   nlaIV
         rsaI                       pleI                               ahaII/bsaHI                  hgiCI
         csp6I                  aciI hinfI                 bsmAI       aatII                        banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                     aluI
                                                                                                    sstI
                                                                                                    sacI
                                                                                                    hgiJII
                                                                                                    hgiAI/aspHI
                                                                                                    ecl136II
                                                                                                    bsp1286
                                                                                                    bsiHKAI
                                                                       rsaI                         bmyI
                          maeIII    aciI       hgaI       aciI         csp6I       mnlI             banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                            haeIII/palI
                                                                                           mcrI
                                                                                           eagI/xmaIII/eclXI
                                                                                           eaeI
                                                                                           cfrI
                                                                                          fnu4HI
                                                                                          aciI
                                                                                         thaI
                      esp3I                                                              fnuDII/mvnI
                 scrFI                                                 sau96I        sacII/sstII
                 mvaI bsmAI                                                 avaII          nspBII
                 ecoRII                                                asuI          kspI  scrFI
                 dsaV                                                 nlaIV          dsaI  ncil
                 bstNI  hinlI/acyI                                        scrFI       bglI bslI  mspI
                 apyI[dcm+]                                               nciI      sau3AI mnlI bstUI
            sau3AI gsuI/bpmI                                              mspI      mboI/ndeII[dam-] hpaII
            mboI/ndeII[dam-]                                    mboII     hpaII     dpnI[dam+] bsaJI dsaV
            dpnI[dam+]  hgaI   fokI                             bpuAI     dsaV      dpnII[dam-] bsh1236I
            dpnII[dam-] ahaII/bsaHI                 mnlI        bbsI      cauII     alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 10C

```
            tfiI
         aciI
        thaI hinfI
        fnuDII/mvnI                                                                                         fnu4HI
        bstUI                                                                                               aciI
        bsh1236I                       aciI                     bstXI                                       thaI
                                  maeII rsaI          pleI scfI  sau96I    styI                             fnuDII/mvnI tru9I
                               maeIII    csp6I    scfI hinfI     haeIII/palI                                bstUI       mseI
                                                                 asuI      bsaJI                            bsh1236I   aseI/asnI/vspI
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC TGCATTCATG GCGGATATCT CAGATATCCG GGTGGGGGAA CCGAAGCAAT CTTGCGCCGA TGTTAATTAT

                                                                                                               sau96I
                                                                                                               avaII
                                                                                                               asuI
                                                                                                            scrFI
                                                                                                            mvaI
                                                                                                            ecoRII
                                                                                                            dsaV
                                                                                                            bstNI
                                   maeIII                                                                   apyI[dcm+]
                                  hphI    scfI         fokI                                           bslI bsaJI
801 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC
    GTATTGGAAT ACATAGTATG TGTATGCTAA ATCCACTGTG ATATCTTATT GTAGGTGAAA CGGAAAGAGA GGTGTCCACA GGTGAGGGTC CAGGTTGACG

                                                   hinlI/acyI
                                                   ahaII/bsaHI
                                                   aatII
                                                 thaI                      taqI[dam-]
                                                 fnuDII/mvnI             sau3AI                    bsmAI
              aluI  taqI                         bstUI                   mboI/ndeII[dam-]        esp3I
    mnlI     hindIII                            aciI maeII               dpnI[dam+]           tth111I/aspI
    bsaJI   ddeI    claI/bsp106              hphI bsh1236I    taqI       dpnII[dam-] taqI     hgaI          sfaNI
901 ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT
    TGGAGCCAAG ATTCGAATAG CTATACTTTT TCGGACTTGA GTGGCGCTGC AGACAGCTCT TCAAAGACTA GCTTTTCAAG CTGTCGCAGA GGCTGGACTA

                                                                                                  hinPI
                                                                                               fnu4HI
     aluI             tfiI                                                                     bbvI
    fnu4HI            hinfI                    taqI                                           aluI  hhaI/cfoI
    bbvI       mnlI  mboII             aluI          mnlI                       aciI
1001 GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA
     CGTCGAGAGC CTCCCGCTTC TTAGAGCACG AAAGTCGAAG CTACATCCTC CCGCACCTAT ACAGGACGCC CATTTATCGA CGCGGCTACC AAAGATGTTT
```

FIG. 10D

```
                                     hinPI
                                     hhaI/cfoI    mspI
                                    thaI          hpaII
                                   aciI          mroI
                                haeIII/palI      bspMII
                               mcrI fnuDII/mvnI  bspEI
     sau3AI                    eagI/xmaIII/eclXI bsaWI
     mboI/ndeII[dam-]          eaeI bstUI      tfiI
     dpnI[dam+]                cfrI bsh1236I   hinfI                 ecoRI
     dpnII[dam-]           sfaNI  fnu4HI  bslI   accIII              apoI                            sfaNI  aciI
1101 GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC
     CTAGCAATAC AAATAGCCGT GAAACGTAGC CGGCGCGAGG GCTAAGGCCT TCACGAACTG TAACCCCTTA AGTCGCTCTC GGACTGGATA ACGTAGAGGG

                                                                                                       mcrI
                                                                                                       eagI/xmaIII/eclXI
                                                                                                       eaeI
                                                                                  styI                fnu4HI
       hgiAI/aspHI                                                          thaI      ncoI       sau3AI  aciI
       bsp1286                                                              fnuDII/mvnI          mboI/ndeII[dam-]
       bsiHKAI                                                        fnu4HI  bstUI     dsaI     dpnI[dam+]      sau3AI
       bmyI                                                           bbvI  mcrI      haeIII/palI dpnII[dam-]    mboI/ndeII[dam-]
       apaLI/snoI                                                    scfI   mspI bsh1236I bsaJI sfaNI   fnu4HI     dpnI[dam+]
       alw44I/snoI    maeII                                nspBII    pstI   hpaII     mnlI nlaIII    pvuI/bspCI haeIII/palI
     bslI draIII    maeIII          bspMI                  aciI      bsgI  cfr10I aciI haeI    fokI  mcrI bbvI cfrI dpnII[dam-]
1201 GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA
     CGGCACGTGT CCCACAGTGC AACGTTGTGG ACGGACTTTG GCTTGACGGG CGACAAGACG TCGGCCAGCG CCTCCGGTAC CTACGCTAGC GACGCCGGCT

                                 sau96I                                                        thaI       sau3AI
                                 avaII                                                         fnuDII/mvnI       nlaIII
                                 asuI                                                          bstUI      mboI/ndeII[dam-]
                         sau96I  rsrII/cspI                                                    bsh1236I   dpnI[dam+]
                 aciI    haeIII/palI  aciI    tfiI                                            hinPI       dpnII[dam-]
      ddeI      bsrBI    asuI       cpoI      hinfI            nlaIII             ndeI hhaI/cfoI  alwI[dam-]
1301 TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG
     AGAATCGGTC TGCTCGCCCA AGCCGGGTAA GCCTGGCGTT CCTTAGCCAG TTATGTGATG TACCGCACTA AAGTATACGC GCTAACGACT AGGGGTACAC

                                                 hinPI                                                          draIII
                                                 hhaI/cfoI                                                     nlaIV
                                                thaI                              bsaJI                        hgiCI
                                                fnuDII/mvnI                     haeIII/palI                    banI
                           tth111I/aspI         bstUI                 sfaNI    sau96I                         mspI
        bsrI             drdI           hgaI    bsh1236I     taqI   aluI       asuI mnlI         bslI         hpaII
1401 TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC
     ATAGTGACCG TTTGACACTA CCTGCTGTGG CAGTCACGCA GGCAGCGCGT CCGAGAGCTA CTCGACTACG AAACCCGGCT CCTGACGGGG CTTCAGGCCG
```

FIG. 10E

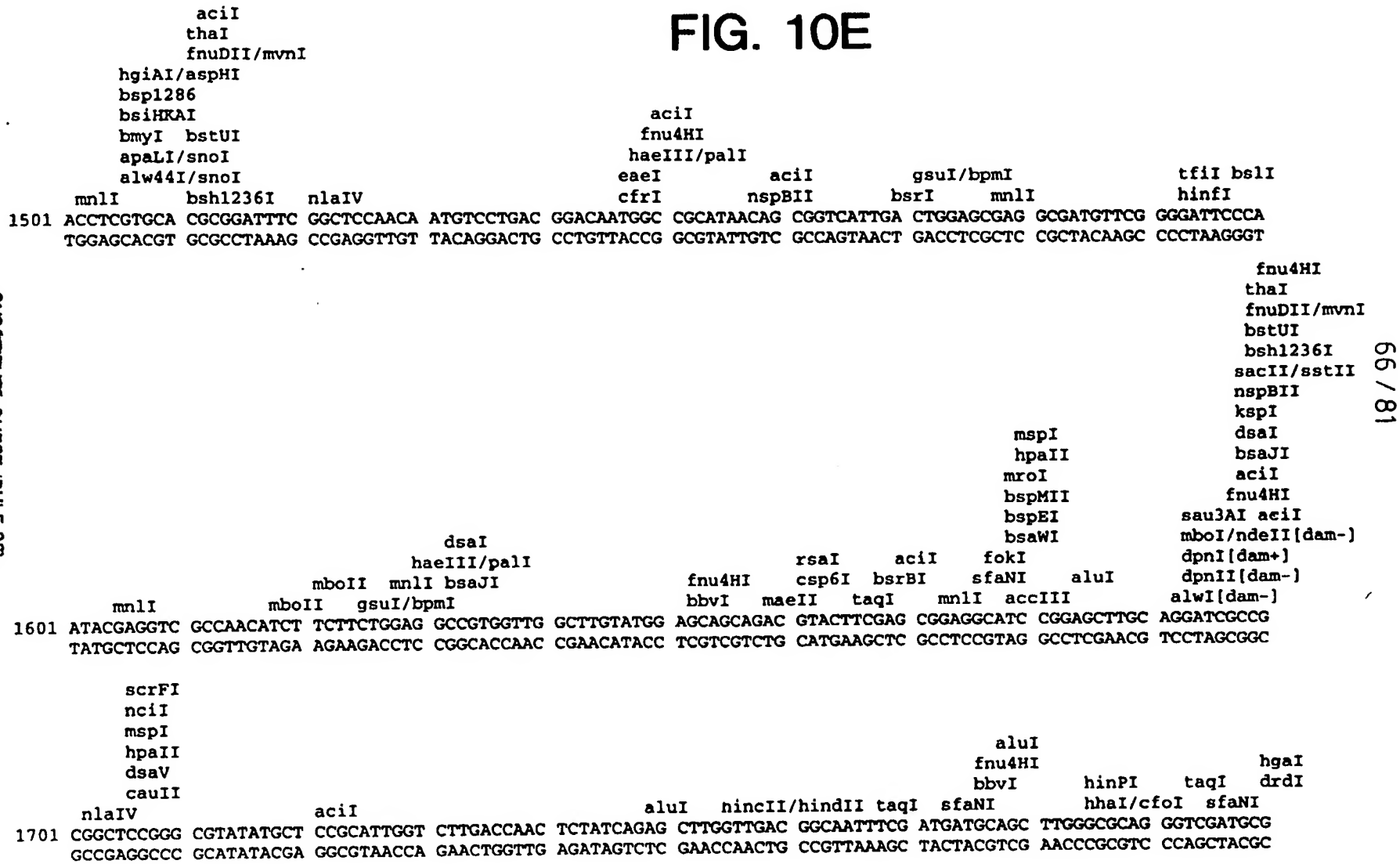

FIG. 10F

```
                       nlaIV
                     mspI                                                haeIII/palI
                     hpaII  scrFI                                       mcrI
                     bslI   nciI                                        eagI/xmaIII/eclXI
                    mroI    mspI                                        eaeI
                    bspMII  hpaII                                       cfrI
                    bspEI[dam-]                                       fnu4HI
                    bsaWI   dsaV                                      aciI
                    accIII[dam-]                                     thaI
                  sau3AI    cauII                                    fnuDII/mvnI
                  mboI/ndeII[dam-]                                   bstUI
                  dpnI[dam+]                                         bsh1236I    sau96I                 rsaI
                  dpnII[dam-]              rsaI                     hinPI        avaII                  csp6I
                  alwI[dam-]               csp6I         aciI       hhaI/cfoI    asuI                  scaI
1801 ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA
     TGCGTTAGCA GGCTAGGCCT CGGCCCTGAC AGCCCGCATG TGTTTAGCGG GCGTCTTCGC GCCGGCAGAC CTGGCTACCG ACACATCTTC ATGAGCGGCT

                                                                                scrFI
                                                                                nciI
                                                                                mspI
                                                                                hpaII
                                                                                dsaV
                                                                               xmaI/pspAI
                                                                               smaI
                                                                               scrFI
                                                                               nciI
                                                                               dsaV
                                                                               cauII
                                                                               bsaJI
                                                                               avaI
                                                                              bsaJI
                                                                           sau3AI                      sau96I
                                                                           mboI/ndeII[dam-]        aciI       haeIII/palI
                                                                           dpnI[dam+]             fnu4HI     asuI
                                                                           dpnII[dam-]            bglI  nlaIII
                                                                           alwI[dam-]            sfiI  styI
                                                                          nlaIV cauII           eaeI   ncoI
                 hinlI/acyI                                     mcrI      bstYI/xhoII           cfrI   dsaI
                 hgaI                 mnlI                     bslI       bamHI bsaJI ecoRI    taqI haeIII/palI
                 ahaII/bsaHI       bsaJI                    sfaNI         alwI[dam-]  apoI    claI/bsp106 bsaJI
1901 TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC
     ATCACCTTTG GCTGCGGGGT CGTGAGCAGG CTCCCGTTTC CTTATCTCAT CTACGGCTGG CTTCCTAGGG GCCCCTTAAG TTAGCTACCG GCGGTACCGG
```

FIG. 10G

```
                     aluI
                   fnu4HI                                                                           rmaI
                   bbvI          maeIII               sfaNI   apoI                             bsmI maeI
2001 CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG
     GTTGAACAAA TAACGTCGAA TATTACCAAT GTTTATTTCG TTATCGTAGT GTTTAAAGTG TTTATTTCGT AAAAAAAGTG ACGTAAGATC AACACCAAAC

                                             sau3AI
                                             mboI/ndeII[dam-]
                                             dpnI[dam+]
                                             dpnII[dam-]
                                           pvuI/bspCI
                                           mcrI
                                          taqI[dam-]   tru9I                 haeIII/palI
                                         claI/bsp106[dam-]                  haeI
                                         sau3AI        mseI       fnu4HI styI
                                         mboI/ndeII[dam-]         bbvI   ncoI
                                         dpnI[dam+] xmnI        hinPI    dsaI
                                         dpnII[dam-]  aseI/asnI/vspI     bsaJI
                                nlaIII  alwI[dam-]  asp700      hhaI/cfoI nlaIII          mnlI      mnlI
2101 TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA
     AGGTTTGAGT AGTTACATAG AATAGTACAG ACCTAGCTAG CCCTTAATTA AGCCGCGTCG TGGTACCGGA CTTTATTGGA GACTTTCTCC TTGAACCAAT

      rsaI                                                                    nlaIV
      csp6I                                                                scrFI                           sfaNI
     nlaIV                                                                 mvaI                                ppu10I
     kpnI                                                                  ecoRII                        nsiI/avaIII
     hgiCI                                                                 dsaV                         nlaIII
     banI                        aluI                                      bstNI                            sphI
     asp718      mnlI           pvuII                                      apyI[dcm+]                       nspI
     acc65I  ddeI   aciI        nspBII                                    bsaJI                             nspHI
2201 GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC
     CCATGGAAGA CTCCGCCTTT CTTGGTCGAC ACCTTACACA CAGTCAATCC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG

                                               nlaIV
                         scrFI        scrFI
                         mvaI              mvaI                                ppu10I
                         ecoRII       ecoRII                        nsiI/avaIII
                         dsaV         dsaV                         nlaIII
                         bstNI             bstNI                             sphI
                         apyI[dcm+]        apyI[dcm+]                        nspI sfaNI
                        sexAI             bsaJI                              nspHI                              aciI
2301 ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC
     TAGAGTTAAT CAGTCGTTGG TCCACACCTT TCAGGGGTCC GAGGGGTCGT CCGTCTTCAT ACGTTTCGTA CGTAGAGTTA ATCAGTCGTT GGTATCAGGG
```

FIG. 10H

```
                                                                                                    fnu4HI
                                                                                                    bglI
                                                                    nlaIII                         sfiI
                                                                   styI                            haeIII/palI
                                                                   ncoI                          mnlI
                 aciI                          bsrI             bslI dsaI                     haeIII/palI
           aciI fokI                      aciI       aciI       aciI bsaJI                  mnlI bsaJI aciI
2401 GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC
     CGGGGATTGA GGCGGGTAGG GCGGGGATTG AGGCGGGTCA AGGCGGGTAA GAGGCGGGGT ACCGACTGAT TAAAAAAAAT AAATACGTCT CCGGCTCCGG

                                                                                       scrFI
                                                                                       nciI
                                                                                       mspI
                                                                                       hpaII
                                                                                       dsaV
                                                              styI                   haeIII/palI
                                                              bsaJI                mcrI
                                                              blnI                 eagI/xmaIII/eclXI
                                                           haeIII/palI        rmaI eaeI
      haeIII/palI                                          stuI rmaI          maeI cfrI
  mnlI     ddeI                            mnlI            haeI maeI         nheI mspI cauII
  bsaJI mnlI  aluI                      mnlI              mnlI avrII       aluI aluI hpaII
2501 GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG
     CGGAGCCGGA GACTCGATAA GGTCTTCATC ACTCCTCCGA AAAAACCTCC GGATCCGAAA ACGTTTTTCG ATCGAATAGG CCGGCCCTTG CCACGTAACC

       tfiI
       hinfI                                                                          fnu4HI
     aciI                                                                             aciI
    thaI                                                         bstXI              thaI
    fnuDII/mvnI                          aciI         scfI  sau96I     styI         fnuDII/mvnI  tru9I
    bstUI                   pleI       rsaI      pleI       haeIII/palI             bstUI        mseI
    bsh1236I                hinfI      csp6I scfI hinfI     asuI       bsaJI        bsh1236I   aseI/asnI/vspI
2601 AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA
     TTGCGCCTAA GGGGCACGGT TCTCAGTCCA TTCATGGCGG ATATCTCAGA TATCCGGGTG GGGGAACCGA AGCAATCTTG CGCCGATGTT AATTATGTAT
```

FIG. 10I

```
                  sau3AI
                  mboI/ndeII[dam-)
                  dpnI[dam+]
                  dpnII[dam-]
                  alwI[dam-]
                taqI[dam-]
              claI/bsp106[dam-]                                                           sau96I
             sau3AI                                                                       avaII
             mboI/ndeII[dam-]                                                             asuI
             dpnI[dam+]                                                                 scrFI
             dpnII[dam-]                                                                mvaI                     rmaI
            alwI[dam-]                                                                  ecoRII         thaI    maeI
                                                                                        dsaV           fnuDII/mvnI
                                                                                        bstNI                bstUI  nheI
                                                                                        apyI[dcm+]    mnlI   bsh1236I  aluI
                                   fokI                                   bslI  bsaJI                 bsaJI  nruI    aluI
2701 ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC
     TGGAAAACCT AGCTAGGATG ACTGTGACTG TAGGTGAAAA AGAAAAAGAG GTGTCCACAG GTGAGGGTCC AGGTTGACGT GGAGCCAAGC GCTTCGATCG

                             nlaIII
                            styI
                            pflMI
                            ncoI
            sfaNI     ecoRI dsaI                                                     rsaI              aluI
         fnu4HI taqI  apoI  bslI fokI                    rmaI               gsuI/bpmI              pvuII tth111I/aspI
         bbvI claI/bsp106   bsaJI       nlaIII   fokI      maeI               bsrI   csp6I      ecoRV nspBII   bsrI
2801 TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA
     AACCCGACGT AGCTAACTTA AGGTGGTACC CTACCAGTAC ATAGTAGGAA AAAGATCATC GTTGACGTTG ACCTCATGTA AGTCTATAGG TCGACTGGGT

           aluI
          sstI
          sacI
          hgiJII
          hgiAI/aspHI
          ecl136II
          bsp1286
          bsiHKAI
          bmyI                          hphI
          banII         mnlI           maeIII   bspMI                 taqI                            bsrI
        avaI        aciI              bstEII  hphI        bsrI      hgaI          hphI   aluI   nlaIII
2901 GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG
     CAGGGGCTCG AGGGACAGGC GGAGACACCC GCTATCCCAG TGGTAGTGGA CGGCACGGTC AGTCTCGCAG CTAATGCTAC CACTATCGAT GTACTTGACC
```

FIG. 10J

```
                                                                                                   mspI
                                                                                                   hpaII
                                                                                                   bslI
                                                           gsuI/bpmI                              bsaWI
                                                           scrFI                           sau3AI
                                                           mvaI                                mboI/ndeII[dam-]
                                                  haeIII/palI                                  dpnI[dam+]
                                          fnu4HI       ecoRII                         dpnII[dam-]
       scrFI                                    aciI         dsaV                          alwI[dam-]
       mvaI                              thaI mnlI     bstNI                              nlaIV
       ecoRII                            fnuDII/mvnI   apyI[dcm+]   pleI                 bstYI/xhoII
       dsaV                                    bstUI       rsaI   pleI gsuI/bpmI              bamHI
       bstNI      aluI                         bsh1236I    csp6I  hinfI     hinfI              alwI[dam-]
       apyI[dcm+]
3001 TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA
     ATAGTTGTCT TTGGTCCTTT TCGAGGCTTT GATGACTAAA TGCGCCGGAG CATGGACCTC AGACCTCAGG GAAGAGCGAA GAGACCTAGG CCAAGACCCT

                                                                                      sau3AI                     styI
                                                                                      mboI/ndeII[dam-]           bsaJI
                                                                                      dpnI[dam+]               rsaI
                                                                                      dpnII[dam-]              csp6I
                                                                                      alwI[dam-]              nlaIV
                                    fnu4HI   mboII                                   nlaIV                    kpnI
                                    bbvI     bpuAI                                   bstYI/xhoII              hgiCI
                                   scfI      bbsI                                    bamHI rsaI               banI
                                   pstI    mspI                                     mnlI   csp6I              asp718
                                   bsgI    hpaII                              maeIII alwI[dam-]               acc65I
3101 CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC
     GCCTAAAGTG AGACTGGTAG TCGTCAGACG TCGGCCTTCT GAAGCGTTGA ATAATGACAG TCGTTTCAGT GCTCCTAGGC ATGTGTAAAC CTGTCCCATG

      sau3AI
      mboI/ndeII[dam-]                        mboII
      dpnI[dam+]             fnu4HI          bpuAI        aciI
      dpnII[dam-]            bbvI            bbsI   mboII                             mnlI
3201 CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
     GTTCCACCTC TAGTTTGCTT GACACCGACG TGGTAGACAG AAGTAGAAGG GCGGTAGACT ACTCGTCAAC TTTAGACCTT GACGGAGACA ACACACGGAC

                                                                                   scrFI
                                                                                   mvaI
                                                                                   ecoRII
                                                                                   dsaV
                                                                                   bstNI
                             haeIII/palI                                           apyI[dcm+]
       xmnI                 haeI      rsaI                  mnlI                              
       asp700              mnlI       csp6I                 bslI      maeIII bsaJI      maeIII
3301 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA
     GACTTATTGA AGATAGGGTC TCTCCGGTTT CATGTCACCT TCCACCTATT GCGGGAGGTT AGCCCATTGA GGGTCCTCTC ACAGTGTCTC GTCCTGTCGT
```

FIG. 10K

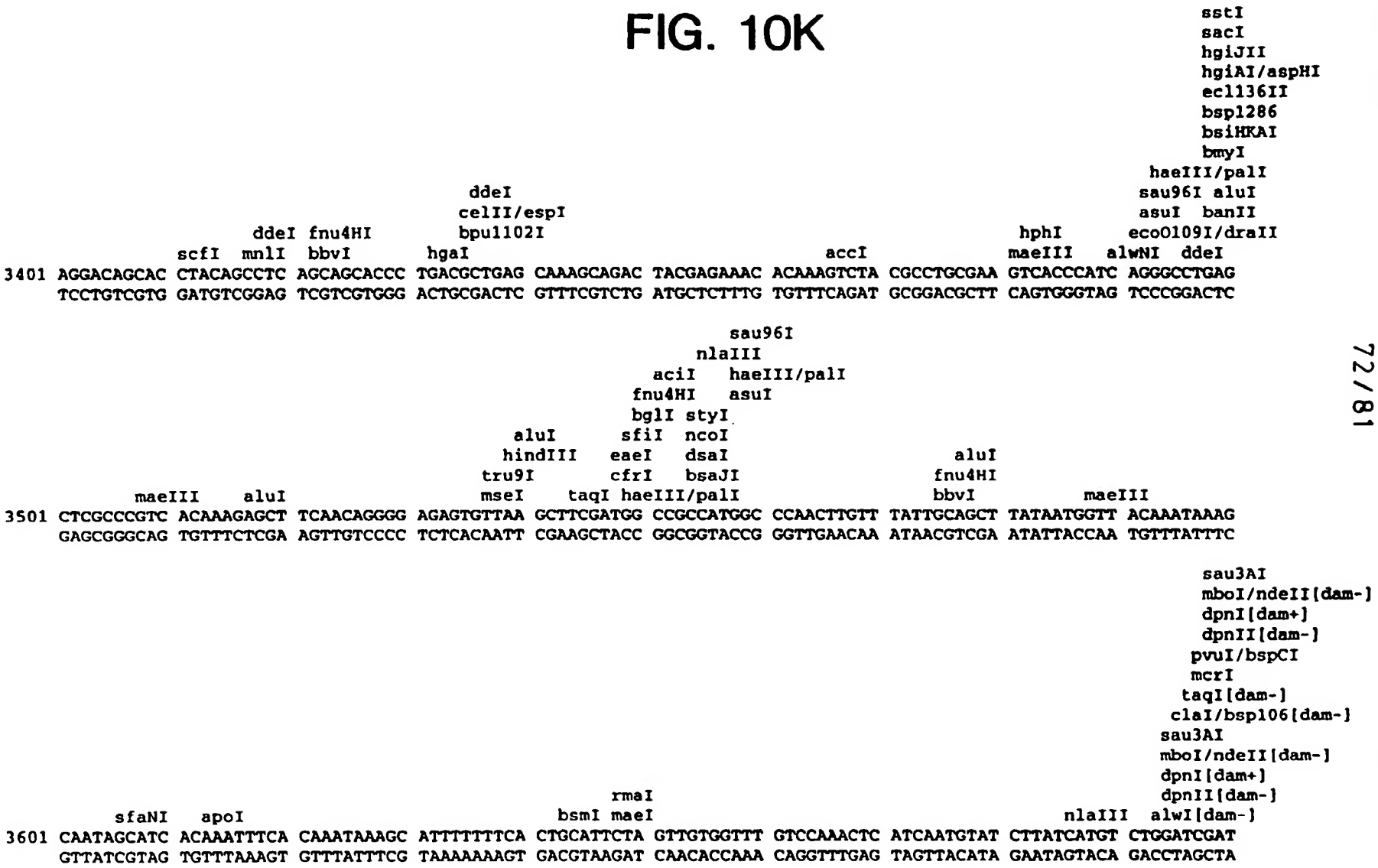

FIG. 10L

```
                                                                         rsaI
                                 haeIII/palI                             csp6I
                                haeI                                    nlaIV
           tru9I      fnu4HI styI                                       kpnI
           mseI       bbvI   ncoI                                       hgiCI
          aseI/asnI/vspI     dsaI                                       banI                        aluI
        xmnI        hinPI    bsaJI                                      asp718     mnlI            pvuII
        asp700      hhaI/cfoI nlaIII         mnlI      mnlI             acc65I  ddeI  aciI         nspBII
3701 CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA AGAACCAGCT GTGGAATGTG
     GCCCTTAATT AAGCCGCGTC GTGGTACCGG ACTTTATTGG AGACTTTCTC CTTGAACCAA TCCATGGAAG ACTCCGCCTT TCTTGGTCGA CACCTTACAC

                                  nlaIV                                                                         nlaIV
                               scrFI                          sfaNI                scrFI        scrFI
                               mvaI                               ppu10I                 mvaI                mvaI
                               ecoRII                       nsiI/avaIII            ecoRII       ecoRII
                               dsaV                        nlaIII                  dsaV         dsaV
                               bstNI                           sphI                      bstNI               bstNI
                               apyI[dcm+]                      nspI                      apyI[dcm+]          apyI[dcm+]
                              bsaJI                            nspHI                    sexAI               bsaJI
3801 TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA AAGTCCCCAG
     ACAGTCAATC CCACACCTTT CAGGGGTCCG AGGGGTCGTC CGTCTTCATA CGTTTCGTAC GTAGAGTTAA TCAGTCGTTG GTCCACACCT TTCAGGGGTC

                                    ppu10I
                                    nsiI/avaIII
                                   nlaIII
                                  sphI
                                  nspI sfaNI                                                                    bsrI
                                  nspHI                               aciI          aciI fokI aciI         aciI
3901 GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC CCGCCCCTAA CTCCGCCCAG
     CGAGGGGTCG TCCGTCTTCA TACGTTTCGT ACGTAGAGTT AATCAGTCGT TGGTATCAGG GCGGGGATTG AGGCGGGTAG GGCGGGGATT GAGGCGGGTC

                                                                    fnu4HI
                                                                    bglI
                         nlaIII                                    sfiI
                        styI                                       haeIII/palI
                        ncoI                                     mnlI    mnlI      ddeI
                   bslI dsaI                                 haeIII/palI bsaJI mnlI  aluI                      mnlI
       aciI        aciI bsaJI                             mnlI bsaJI aciI    haeIII/palI                    mnlI
4001 TTCCGCCCAT TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC
     AAGGCGGGTA AGAGGCGGGG TACCGACTGA TTAAAAAAAA TAAATACGTC TCCGGCTCCG GCGGAGCCGG AGACTCGATA AGGTCTTCAT CACTCCTCCG
```

FIG. 10M

```
                styI                                                                                 scrFI
                bsaJI                                                                                mvaI
                blnI                                                                                 ecoRII
             haeIII/palI                  tru9I            haeIII/palI                               dsaV
            stuI rmaI                     mseI            eaeI                                       bstNI
            haeI maeI                    hpaI  aluI       cfrI                     maeIII            apyI[dcm+]        tru9I
           mnlI avrII               aluI hincII/hindII  bsrI                  maeII    bsrI         bsaJI  maeIII     mseI
4101 TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG CTGTTAACAG CTTGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT
     AAAAAACCTC CGGATCCGAA AACGTTTTTC GACAATTGTC GAACCGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT TTGGGACCGC AATGGGTTGA

                                                                           sau3AI
                                                                           mboI/ndeII[dam-]
                                                                 sau96I    dpnI[dam+]
                                                                 haeIII/palI
                                               aluI           mnlI  aciI   dpnII[dam-]
           fnu4HI                             pvuII         mboII asuI    pvuI/bspCI
           bbvI  fokI                         nspBII        earI/ksp632I  mcrI                bglI
4201 TAATCGCCTT GCAGCACATC CCCCCTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGTAGCCT GAATGGCGAA
     ATTAGCGGAA CGTCGTGTAG GGGGGAAGCG GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCATCGGA CTTACCGCTT

      hinPI
      hhaI/cfoI
     nlaIV                                                                            hinPI
     narI                                                                             hhaI/cfoI
     kasI                                                                            thaI
     hinlI/acyI                                                                      fnuDII/mvnI              hinPI
     hgiCI                                                                           bstUI          hinPI    hhaI/cfoI
     haeII      aciI                                                                 bsh1236I       hhaI/cfoI
     banI   sfaNI                                                                 rsaI       scfI fnu4HI    tru9I
     ahaII/bsaHI                         sfaNI    aciI           aciI  maeII      csp6I     bslI  aciI     mseI
4301 TGGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTCACA CCGCATACGT CAAAGCAACC ATAGTACGCG CCCTGTAGCG GCGCATTAAG
     ACCGCGGACT ACGCCATAAA AGAGGAATGC GTAGACACGC CATAAAGTGT GGCGTATGCA GTTTCGTTGG TATCATGCGC GGGACATCGC CGCGTAATTC

                          fnu4HI
        aciI            hinPI
      fnu4HI            hhaI/cfoI                            hinPI
      aciI             thaI                                  hhaI/cfoI
     thaI              fnuDII/mvnI                          haeII
     fnuDII/mvnI       bstUI                          hinPI rmaI
     bstUI             bsh1236I    aciI               hhaI/cfoI   bsrBI
     bsh1236I     maeIII  bbvI maeIII                haeII  maeI   aciI          mboII                maeII
4401 CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT CGCCACGTTC
     GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA GCGGTGCAAG
```

FIG. 10N

```
                                          nlaIV                                          mnlI
      mspI                                hgiJII                                    nlaIV
      hpaII                               bsp1286                                   hgiCI
     naeI                                 bmyI                                      banI   taqI                   hphI
     cfr10I             aluI              banII           nlaIV
4501 GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TTGGGTGATG
     CGGCCGAAAG GGGCAGTTCG AGATTTAGCC CCCGAGGGAA ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT TTTTGAACTA AACCCACTAC

       maeII    haeIII/palI
      draIII   sau96I                                     maeII  pleI        tru9I      pleI
      bsaAI    asuI                                       drdI   hinfI maeII mseI       hinfI          bsrI
4601 GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC
     CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT GACCTTGTTG

                                                                                                            thaI
                                                                                                            fnuDII/mvnI
                                                                                                            bstUI
                                                                        tru9I                               bsh1236I
        bslI                                                            mseI              tru9I     tru9I
       bslI    avaI                                           haeIII/palI         aluI    mseI  apoI mseI    apoI
4701 ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG
     TGAGTTGGGA TAGAGCCCGA TAAGAAAACT AAATATTCCC TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT AAATTGTTTT TAAATTGCGC

                                             hgiAI/aspHI
                                             bsp1286
                       maeII                 bsiHKAI                                                          tth111I/aspI
                     tru9I                   bmyI    ddeI                   aciI                             maeIII
       tru9I         mseI                    apaLI/snoI  rsaI             fnu4HI    tru9I                   maeII
       mseI     sspI   psp1406I              alw44I/snoI csp6I           sfaNI      mseI        aciI        bsaAI bsrI
4801 AATTTTAACA AAATATTAAC GTTTACAATT TTATGGTGCA CTCTCAGTAC AATCTGCTCT GATGCCGCAT AGTTAAGCCA ACTCCGCTAT CGCTACGTGA
     TTAAAATTGT TTTATAATTG CAAATGTTAA AATACCACGT GAGAGTCATG TTAGACGAGA CTACGGCGTA TCAATTCGGT TGAGGCGATA GCGATGCACT

                                                    hinPI
                                                    hhaI/cfoI                  mspI
                                                   thaI                        hpaII
          hinPI                                    fnuDII/mvnI                scrFI                             bsmAI
        fnu4HI                                     bstUI                      nciI    aciI                     esp3I
        bbvI                              nspBII   bsh1236I                   dsaV sfaNI                       bslI
      nlaIII  hhaI/cfoI    aciI           aciI   hgaI        drdI             cauII fokI             aluI maeIII
4901 CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC
     GACCCAGTAC CGACGCGGGG CTGTGGGCGG TTGTGGGCGA CTGCGCGGGA CTGCCCGAAC AGACGAGGGC CGTAGGCGAA TGTCTGTTCG ACACTGGCAG
```

FIG. 100

```
                                                         thaI
                                                         fnuDII/mvnI
                                                         bstUI
                                                         bsh1236I
     scrFI                                              hinPI
     nciI                                               hhaI/cfoI                           mnlI
     mspI      nspI                                     thaI                               haeIII/palI
     hpaII fnu4HI                                       fnuDII/mvnI          mboII        sau96I
     dsaV aluI nspHI                                    bstUI mnlI           bpuAI        asuI
     cauII bbvI nlaIII    mnlI   hphI       hphI        bsh1236I             bbsI        eco0109I/draII
5001 TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGTAT TCTTGAAGAC GAAAGGGCCT CGTGATACGC CTATTTTTAT
     AGGCCCTCGA CGTACACAGT CTCCAAAAGT GGCAGTAGTG GCTTTGCGCG CTCCGTCATA AGAACTTCTG CTTTCCCGGA GCACTATGCG GATAAAAATA

                                                                      nlaIV
                                                                    aciI
                                                                   thaI
                                                                   fnuDII/mvnI
                                          maeII                    bstUI
             nlaIII                      hinlI/acyI                bsh1236I
       tru9I rcaI                        ahaII/bsaHI              hinPI
       mseI  bspHI                   ddeI aatII                   hhaI/cfoI
5101 AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC
     TCCAATTACA GTACTATTAT TACCAAAGAA TCTGCAGTCC ACCGTGAAAA GCCCCTTTAC ACGCGCCTTG GGGATAAACA AATAAAAAGA TTTATGTAAG

                 rcaI
                 bspHI
             bsrBI   bsmAI                                       mboII
             aciI nlaIII                            sspI         earI/ksp632I
5201 AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT
     TTTATACATA GGCGAGTACT CTGTTATTGG GACTATTTAC GAAGTTATTA TAACTTTTTC CTTCTCATAC TCATAAGTTG TAAAGGCACA GCGGGAATAA

                                                                                        hgiAI/aspHI
                                                                                        bsp1286
                                                                               sau3AI   bsiHKAI
                                                                               mboI/ndeII[dam-]
                                                                               dpnI[dam+] bmyI
                                                                               dpnII[dam-]
            fnu4HI                                                       eco57I         apaLI/snoI
            aciI                   hphI          hphI               sfaNI mboII[dam-]   alw44I/snoI  maeIII
5301 CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA
     GGGAAAAAAC GCCGTAAAAC GGAAGGACAA AAACGAGTGG GTCTTTGCGA CCACTTTCAT TTTCTACGAC TTCTAGTCAA CCCACGTGCT CACCCAATGT
```

FIG. 10P

```
            sau3AI
            mboI/ndeII[dam-]    sau3AI
            dpnI[dam+]          mboI/ndeII[dam-]
            dpnII[dam-]         dpnI[dam+]                    psp1406I         hgiAI/aspHI                  aciI
           bstYI/xhoII          dpnII[dam-]                   xmnI             bsp1286  tru9I               thaI
       bsrI          nspBII     alwI[dam-]                    asp700           bsiHKAI  mseI                fnuDII/mvnI
   taqI   alwI[dam-]  aciI     bstYI/xhoII                mboII maeII          bmyI     ahaIII/draI         bstUI
                                                                                                            bsh1236I
                                                                                                            hinPI
                                                                                                            hhaI/cfoI
5401 TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT
     AGCTTGACCT AGAGTTGTCG CCATTCTAGG AACTCTCAAA AGCGGGGCTT CTTGCAAAAG GTTACTACTC GTGAAAATTT CAAGACGATA CACCGCGCCA

                   scrFI
                   nciI
                   mspI
                   hpaII
                   dsaV
                   cauII
               hinlI/acyI                                                        rsaI
               hgaI                     aciI                                     csp6I  bsrI
               ahaII/bsaHI    bcgI   mcrI fnu4HI        ddeI                     scaI hphI   maeIII     sfaNI
5501 ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT
     TAATAGGGCA CTACTGCGGC CCGTTCTCGT TGAGCCAGCG GCGTATGTGA TAAGAGTCTT ACTGAACCAA CTCATGAGTG GTCAGTGTCT TTTCGTAGAA

                                                                                                      sau96I
                                                                                                      avaII
                                                                                              sau3AI  asuI
                                                                         haeIII/palI          mboI/ndeII[dam-]
                                                                         eaeI                 dpnI[dam+]
                                                                         cfrI                 dpnII[dam-]
                                       fnu4HI                            fnu4HI               pvuI/bspCI
  fokI  nlaIII                         bbvI          nlaIII              aciI                 mcrI  mnlI
5601 ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG
     TGCCTACCGT ACTGTCATTC TCTTAATACG TCACGACGGT ATTGGTACTC ACTATTGTGA CGCCGGTTGA ATGAAGACTG TTGCTAGCCT CCTGGCTTCC

                                     nlaIII
                                 sau3AI maeIII                          mspI
                                 mboI/ndeII[dam-]      sau3AI    nlaIV
                                 dpnI[dam+]            mboI/ndeII[dam-]
                                alwI[dam-]             dpnI[dam+]    hpaII
alul    aciI               nlaIII dpnII[dam-]          dpnII[dam-] bsaWI aluI                                 maeIII
5701 AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC
     TCGATTGGCG AAAAAACGTG TTGTACCCCC TAGTACATTG AGCGGAACTA GCAACCCTTG GCCTCGACTT ACTTCGGTAT GGTTTGCTGC TCGCACTGTG
```

FIG. 10Q

```
                                                                                   mspI
                        hinPI                                                      hpaII
                      mstI                                                       scrFI
                      aviII/fspI      bsrI                                 aluI nciI        tru9I      fokI
          fnu4HI    maeII hhaI/cfoI  tru9I                                 rmaI dsaV        mseI     bsrI       aciI
     sfaNI   bbvI        psp1406I     mseI                                 maeI cauII     aseI/asnI/vspI   mnlI
5801 CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG
     GTGCTACGGT CGTCGTTACC GTTGTTGCAA CGCGTTTGAT AATTGACCGC TTGATGAATG AGATCGAAGG GCCGTTGTTA ATTATCTGAC CTACCTCCGC

                                                                                                aciI
                        bglI                                                    mspI          thaI
                      sau96I                                                    hpaII         fnuDII/mvnI
           sau96I     haeIII/palI                                             cfr10I          bstUI
           avaII    hinPI asuI    mspI                                     nlaIV  hphI     bsmAI          fnu4HI
           asuI     hhaI/cfoI     hpaII                                   gsuI/bpmI        bsaI bsh1236I  bbvI
5901 GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG
     CTATTTCAAC GTCCTGGTGA AGACGCGAGC CGGGAAGGCC GACCGACCAA ATAACGACTA TTTAGACCTC GGCCACTCGC ACCCAGAGCG CCATAGTAAC

                                                                                                    ddeI
        sau96I                                                                                   sau3AI
        asuI                                             pleI                                    mboI/ndeII[dam-]
       nlaIV                                             hinfI                                   dpnI[dam+]
     bsrI haeIII/palI     mnlI                       eam1105I              fokI                  dpnII[dam-]
6001 CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT
     GTCGTGACCC CGGTCTACCA TTCGGGAGGG CATAGCATCA ATAGATGTGC TGCCCCTCAG TCCGTTGATA CCTACTTGCT TTATCTGTCT AGCGACTCTA

                                                                                                      hphI
                                                                                                    rmaI
                                                                                                 sau3AI
                                                                                                 mboI/ndeII[dam-]
                                                                                                 dpnI[dam+]
       mnlI                                                                               tru9I  dpnII[dam-]
     nlaIV                                                                    tru9I     ahaIII/draI maeI
     hgiCI       tru9I                                                        mseI     tru9I     bstYI/xhoII
     banI        mseI      maeIII                                           ahaIII/draI mseI mseI alwI[dam-]
6101 AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG
     TCCACGGAGT GACTAATTCG TAACCATTGA CAGTCTGGTT CAAATGAGTA TATATGAAAT CTAACTAAAT TTTGAAGTAA AAATTAAATT TTCCTAGATC
```

FIG. 10R

```
   sau3AI
   mboI/ndeII[dam-]
   dpnI[dam+]
   dpnII[dam-]
   alwI[dam-]                 nlaIII                maeII
   bstYI/xhoII                rcaI              tru9I
  mboII[dam-]                 bspHI             mseI                       ddeI hgaI
6201 GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT
     CACTTCTAGG AAAAACTATT AGAGTACTGG TTTTAGGGAA TTGCACTCAA AAGCAAGGTG ACTCGCAGTC TGGGGCATCT TTTCTAGTTT CCTAGAAGAA
```

Additional sites for 6201 (right end): sau3AI, mboI/ndeII[dam-], dpnI[dam+], dpnII[dam-], bstYI/xhoII, alwI[dam-]; sau3AI, mboI/ndeII[dam-], dpnI[dam+] mboII[dam-], dpnII[dam-]

```
                    thaI                                                             sau3AI
  sau3AI            fnuDII/mvnI                                                      mboI/ndeII[dam-]
  mboI/ndeII[dam-]                                                                   dpnI[dam+]
  dpnI[dam+]        bstUI                                                            dpnII[dam-]
  dpnII[dam-]       bsh1236I                                                        alwI[dam-]
  alwI[dam-]       hinPI       fnu4HI                              aciI            mspI
 bstYI/xhoII       hhaI/cfoI   bbvI                         aciI   nspBII          hpaII       aluI
6301 GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAAACCA CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT
     CTCTAGGAAA AAAAGACGCG CATTAGACGA CGAACGTTTG TTTTTTTGGT GGCGATGGTC GCCACCAAAC AAACGGCCTA GTTCTCGATG GTTGAGAAAA

          bsrI           hinPI                      rmaI           haeIII/palI
      maeIII    eco57I   hhaI/cfoI                  maeI     bslI  haeI                   scfI     aciI
6401 TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT
     AGGCTTCCAT TGACCGAAGT CGTCTCGCGT CTATGGTTTA TGACAGGAAG ATCACATCGG CATCAATCCG GTGGTGAAGT TCTTGAGACA TCGTGGCGGA

                                                                  scrFI
                                                                  nciI
                                     fnu4HI                       mspI                         mspI
                             alwNI   bbvI                         hpaII                        hpaII
                            bsrI  fnu4HI                          dsaV      pleI             bsaWI       hinPI
    mnlI                 maeIII   bbvI      bsrI                  cauII     hinfI          maeIII        hhaI/cfoI
6501 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG
     TGTATGGAGC GAGACGATTA GGACAATGGT CACCGACGAC GGTCACCGCT ATTCAGCACA GAATGGCCCA ACCTGAGTTC TGCTATCAAT GGCCTATTCC

                             hgiAI/aspHI
    mcrI                     bsp1286
   aciI                      bsiHKAI
  nspBII                     bmyI                                                                    hinPI
 fnu4HI                      apaLI/snoI                                                              hhaI/cfoI
 bbvI                        alw44I/snoI   aluI                       ddeI      scfI                haeII
6601 CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG
     GCGTCGCCAG CCCGACTTGC CCCCCAAGCA CGTGTGTCGG GTCGAACCTC GCTTGCTGGA TGTGGCTTGA CTCTATGGAT GTCGCACTCG TAACTCTTTC
```

FIG. 10S

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                                                                                                          scrFI
                                                                                                          mvaI         scrFI
                                                                                                          ecoRII  mvaI
                                                 mspI                                                     dsaV    ecoRII
                                                 hpaII                                                    bstNI        dsaV
                                                 bslI    fnu4HI                        hinPI  mnlI        bsaJI        bstNI
                                        aciI     bsaWI   aciI                          hhaI/cfoI   aluI   apyI[dcm+]   apyI[dcm+]
6701 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG
     GCGGTGCGAA GGGCTTCCCT CTTTCCGCCT GTCCATAGGC CATTCGCCGT CCCAGCCTTG TCCTCTCGCG TGCTCCCTCG AAGGTCCCCC TTTGCGGACC

                                                                                                                fnu4HI
                                                                                                                 aciI
                                                                                                                thaI
                                                                                                               fnuDII/mvnI
                                                         taqI                              nlaIV               bstUI
                                           mnlI  drdI    hgaI          sfaNI              aciI                 bsh1236I
6801 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG
     ATAGAAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCGCAGCTAA AAACACTACG AGCAGTCCCC CCGCCTCGGA TACCTTTTTG CGGTCGTTGC

                haeIII/palI
              scrFI
              mvaI
              ecoRII
              dsaV                   nlaIII
              bstNI bslI                 nspI
   bslI       apyI[dcm+]      haeIII/palI nspHI                      tfiI
  haeIII/palI nlaIV haeI    haeI         aflIII                      hinfI                  aciI
6901 CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA
     GCCGGAAAAA TGCCAAGGAC CGGAAAACGA CCGGAAAACG AGTGTACAAG AAAGGACGCA ATAGGGGACT AAGACACCTA TTGGCATAAT GGCGGAAACT
```

FIG. 10T

```
                                                                                                                thaI
                                                                                                                fnuDII/mvnI
                                                                                                                bstUI
                                                                                                                bsh1236I
                                                                                                               hinPI
                                                                                                               hhaI/cfoI
                                                                                                              thaI
                                                                                hinPI                         fnuDII/mvnI
                           fnu4HI             fnu4HI                       sapI hhaI/cfoI                     bstUI
                           bbvI               bbvI  pleI                   mboII                              bsh1236I
                 bsrBI  aciI                hinPI   hinfI                  earI/ksp632I              mnlI    bslI
        aluI     aciI  fnu4HI       mcrI    hhaI/cfoI            mnlI   aciI   haeII              aciI       aciI
7001 GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG
     CACTCGACTA TGGCGAGCGG CGTCGGCTTG CTGGCTCGCG TCGCTCAGTC ACTCGCTCCT TCGCCTTCTC GCGGGTTATG CGTTTGGCGG AGAGGGGCGC

                    tru9I   aluI
         haeIII/palI       pvuII                                                     tru9I                      nlaIV
        eaeI tfiI   mseI   nspBII                                        hinPI       mseI      maeIII           hgiCI
        cfrI hinfI aseI/asnI/vspI                   bsrI     aciI        hhaI/cfoI  aseI/asnI/vspI mnlI         banI
7101 CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG
     GCAACCGGCT AAGTAATTAG GTCGACCGTG CTGTCCAAAG GGCTGACCTT TCGCCCGTCA CTCGCGTTGC GTTAATTACA CTCAATGGAG TGAGTAATCC

         scrFI
         mvaI
         ecoRII
         dsaV
         bstNI
         apyI(dcm+)              mspI                          aciI                                             xmnI
        bsaJI                    hpaII                       bsrBI                          aluI     nlaIII     asp700
7201 CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA
     GTGGGGTCCG AAATGTGAAA TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT GTGTCCTTTG TCGATACTGG TACTAATGCT

      tru9I
      mseI
     aseI/asnI/vspI
7301 ATTAA
     TAATT

>length: 7305
```

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCI/US 95/09576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/64 C12N15/67 C12N15/85 C12N9/72 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DNA CLONING, VOLUME III, EDITED BY D.M. GLOVER, 1987 IRL PRESS, OXFORD, GB;, pages 189-212, A.M.C. BROWN AND M.R.D. SCOTT 'Retroviral vectors'	1-3,7,8
Y	see page 192, line 7 - page 196, line 5; figures 2,3	5,6, 9-12, 16-21
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	CELL, vol. 37, no. 3, July 1984 CELL PRESS,CAMBRIDGE,MA,US;, pages 1053-1062, C.L. CEPKO ET AL. 'Construction and applications of a highly transmissible murine retrovirus shuttle vector' cited in the application	1-3,7,8
Y	pZIP-Neo SV(B)1 see figure 1	5,6, 9-12, 16-21

Y	MOL. CELL. BIOL., vol. 5, no. 3, March 1985 ASM WASHINGTON, DC,US, pages 431-437, A.D. MILLER ET AL. 'Generation of helper-free amphotrophic retroviruses that tranduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene' see page 432, right column, line 5 - page 436, right column, line 7; figure 1	5,6, 9-12, 16-21

Y	WO,A,94 05784 (US) 17 March 1994 see the whole document	5,6, 9-12, 16-21

Y	EP,A,0 215 548 (ZYMOGENETICS INC ;UNIV WASHINGTON (US)) 25 March 1987 see the whole document	5,6, 9-12, 16-21

A	WO,A,92 17566 (GENENTECH INC) 15 October 1992 cited in the application see the whole document	1-21

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROC. NATL.ACAD SCI., vol. 86, February 1989 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 1041-1045, M. VIVAUD ET AL. 'A 5' splice-region G-C mutation in exon 1 of the human beta-globin gene inhibits pre-mRNA splicing: A mechanism for beta+-thalassemia' see the whole document	1-4

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 95/09576

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